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NATURE OF INJURY TO FORAGE LEGUMES BY THE POTATO LEAF HOPPER[1]

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INTRODUCTION

Since 1927, when it was first shown that the yellowing of alfalfa, *Medicago sativa* L. (*14*),[3] and the reddening, yellowing, and browning of red clover, *Trifolium pratense* L. (*13*), were caused by the potato leaf hopper, *Empoasca fabae* (Harris), there has been considerable speculation concerning the immediate cause and the nature of the pathological disturbance within the infested plants. The similarity of these symptoms on legumes to those of the yellows diseases of plants caused by insect-transmitted viruses led to suggestions that this might be another such disease. However, evidence was soon produced that the symptoms were not due to a virus, and several workers inclined toward the theory that the insect when feeding injects into the plant tissue some chemical or enzymic toxin. Recent additions to knowledge concerning the feeding habits of this insect, the histological changes it causes in the plants it feeds upon, and the chemical changes that follow the feeding injury indicate, however, that although the injection of a toxic substance during feeding is possible it is not highly probable.

REVIEW OF LITERATURE

The evidence in the literature regarding the injection of a specific virus or toxin by this leaf hopper during feeding is contradictory. Ball (*2*) in 1919 reported that the hopperburn it caused on potato was specific, i.e., caused by only one species of leaf hopper, and advanced the idea that it was an insect-transmitted trouble, somewhat similar to curly top of sugar beets. Fenton and Ressler (*7*) in 1922 reported the artificial production of a leaf necrosis very similar to hopperburn on potato by injecting leaf-hopper extract into healthy leaves. In their tests, inoculum prepared from macerated adults produced more

[1] Received for publication Feb. 24, 1934; issued September 1934. This paper reports the results of certain phases of a cooperative study of the injury by *Empoasca fabae* (Harris) to forage legumes being made by the Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] The writer is indebted to F. W. Poos, of the Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, for his cooperation in supplying the leaf hoppers used in the studies here reported; also to F. F. Smith, of the Bureau of Entomology and Plant Quarantine, for the use of small feeding cages constructed by him, and for transferring the cages and leaf hoppers from petiole to petiole of experimental plants. The writer is also indebted to R. G. Capen and M. R. Coe, of the Food Research Division, Bureau of Chemistry and Soils, U.S. Department of Agriculture, for analyzing the 1931 samples. The 1932 analytical work was done at the Arlington Experiment Farm, Rosslyn, Va., in the fruit production laboratory of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry. Thanks are due J. R. Magness for supplying laboratory facilities and equipment and L. O. Regiembal for many helpful suggestions.

[3] Reference is made by number (italic) to Literature Cited, p. 404.

injury than that from the nymphs. The same year, Eyer (*5*) reported that he had been able to produce hopperburn by injecting an extract of leaf hoppers, especially of the nymphs, into healthy plants, and that he had reinoculated the "specific" from diseased plants into healthy plants and had again produced the characteristic burning. However, in his tests the action of the sun's rays was necessary for the browning of the leaves after the yellowing had been produced by inoculation.

In 1923 Fenton and Hartzell (*6*) reported further attempts to produce potato hopperburn artificially. They had failed to produce any injury comparable to hopperburn by mutilation of the leaf tissue with various instruments, by the injection of dilute poisons into healthy leaves, or by inoculations with solutions prepared from hopperburn-diseased tissue. Positive results were obtained, however, from a few of their inoculations with aqueous and alcoholic extracts from macerated adult leaf hoppers. When nymphs were used the results were all negative. In spite of their variable results, these writers concluded that there was some specific substance in the insect's body that could be dissolved in either water or dilute alcohol by maceration, and that if enough of this was injected into the vascular system of the leaf a form of injury resulted which closely resembled that produced by the feeding of the insects. Lutman (*16*) in 1923 expressed the opinion that the evidence for the injection of a specific toxin or inciting agent by the leaf hopper was not conclusive, and advanced the theory that in sucking the juice from the veins of the potato leaves the hoppers—

> remove the water from the tubes and from the adjacent sieve tubes, companion cells and elongated parenchyma to such a degree that they plasmolyze and collapse to a point where they cannot regain their turgidity, and, as a consequence, they die and become brown in color.

Granovsky (*8*) in 1926 described a marginal yellowing and reddening of apple leaves caused by this leaf hopper and suggested the existence of some infective principle (virus) localized in the vascular system of the leaves. He reported artificial production of the symptoms by needle inoculations with inoculum prepared from macerated adults and advanced nymphal stages of the insect, by crushing nymphs and adults on apple leaves, and in a few cases by needle inoculations with the sap of discolored leaves.

In 1930 Smith and Barker (*30*) described a yellows disease of beans in Haiti caused by *Empoasca fabalis* De Long, a new species of *Empoasca* closely related to *E. fabae*, and reported that it could not be transmitted by the inoculation of healthy plants with juice from diseased foliage. So far as the writer can determine, this is the only reported attempt to transmit an *Empoasca* yellowing on legumes by artificial inoculation.

Fenton and Hartzell (*6*) in 1923 reported that the hopperburn produced by *E. fabae* on potato was localized to the part of the plant upon which the leaf hoppers were confined and that the amount of hopperburn produced was directly correlated with the number of hoppers present on the plants. These facts were soon confirmed for the injury produced by this leaf hopper on forage legumes, and it was observed further that plants which were not too severely injured recovered if the hoppers were kept off. There appeared to be little reason, therefore, for assuming that a virus was concerned in the production of the symptoms on legumes, except for the resemblance of

the symptoms of this leaf-hopper injury to those of other yellows diseases of plants reported to be caused by insect-transmitted viruses. Consequently, Granovsky (*9*), Monteith and Hollowell (*20*), and Poos (*21*) all suggested that the probable cause was a toxin or enzyme injected by the leaf hopper into the tissue of the plants and remaining more or less localized.

Poos (*21*) in 1929 summarized as follows the facts established by the earlier workers and himself which tended in his opinion to support the theory that the insect when feeding injects into the plant tissue some chemical or enzymic toxin. (1) The amount of injury produced by *Empoasca fabae* was apparently directly proportional to the number of these insects which were present. (2) When *E. fabae* was removed from a slightly injured plant, the plant invariably recovered its natural growth. (3) It was not possible to rear or collect any individual of *E. fabae* that was free from the infectious principle. (4) A transfer of *E. fabae* on various host plants seemed to show no difference in the degree of the injurious symptoms or in the period of time required to produce them when the insects were again placed on seedlings of clover and alfalfa. (5) A comparison of the capacity for injury of the various instars of *E. fabae* with that of the adult showed little difference in the type and degree of injury caused by the various stages. (6) The diseaselike symptoms were not found to be systemic. In both alfalfa and clover, when shoots of the same plant were caged separately, with one cage containing *E. fabae* while another was left uninfested as a check, the shoots in the infested cages showed the typical injury whereas those in the check cages always grew normally.

Granovsky (*10*) in 1930 reported two observations which threw light on the nature of the injury caused by the potato leaf hopper to alfalfa: (1) Histological study of the injured tissue showed a clogging and isolation of the vascular bundles by what he considered to be suberized or lignified layers, and complete disorganization of the phloem tissue in some cases; and (2) microchemical tests showed a greater accumulation of starch grains and reducing sugars in the tissue injured by the leaf hopper. Smith and Poos (*29*) and Smith (*28*) have since reported that the clogging material is largely of insect origin and responds positively to microchemical tests for proteins.

In 1931 Smith and Poos (*29*) reported that histological study of the feeding habits of *Empoasca fabae* and five closely related species of *Empoasca* had shown the following results. (1) Of the species studied only *E. fabae* fed upon the vascular tissues of the plants and caused a disorganization and plugging of the conducting elements. The other five species, distinguishable from *E. fabae* only by examination of the structures in the male genital chamber, fed upon the mesophyll of the leaves and caused only a whitish spotting or stippling where mesophyll cells were emptied of their contents. (2) Serial sections of a cowpea petiole on which an adult *E. fabae* had fed for 24 hours showed that 14 punctures had been made into the phloem and that 5 of the 7 bundles in the petiole had been fed upon. (3) The injury produced by *E. fabae*, whether wilting or reddening and yellowing, was localized to the part of the plant beyond the point of feeding. These workers concluded from their histological studies that injury by the potato leaf hopper is not due to the introduction of a toxic substance into the plant but is the result of interference with the translocation of plant materials.

HYPOTHESIS OF OVERACCUMULATION OF CARBOHYDRATES

The writer started work on certain phases of this cooperative project in 1930, when the histological work of F. F. Smith and F. W. Poos was in progress. Through their cooperation and by means of the small feeding cages constructed by Smith (*27*), he first undertook a detailed study of the reaction of leaves of Ladino clover, *Trifolium repens* L. var. *latum* McCarthy, to the feeding injury caused by individual leaf hoppers confined upon restricted areas of the leaf petioles for 24 hours. Ladino clover was selected as the host plant for use in this work because of its extreme susceptibility to the injury and because its ease of propagation by shoot cuttings made it possible to secure abundant, uniform host material. The leaf hoppers used in these tests were from a Virginia strain of *Empoasca fabae* established by Dr. Poos in 1928 and

FIGURE 1.—Wilting of Ladino clover leaves 3 to 7 days after individual *Empoasca fabae* had fed upon restricted areas of the petioles about 1 inch from the blades for 24 hours. On this host collapse of the region of the petiole fed upon precedes the complete wilting of the blade. The two lower leaves are uninjured checks from the same plants as the leaves above.

maintained continuously by him on cowpeas in cheesecloth cages in the greenhouse. During the feeding exposure the plants were kept in a greenhouse at 80° F., a temperature favorable for active leaf-hopper feeding, but as soon as the leaf hoppers were removed at the end of the feeding period the plants were taken to another greenhouse, where the temperature was maintained between 50° and 55°. At this low temperature the reddening and yellowing developed slowly, and an excellent opportunity was afforded to study their gradual increase in intensity. Figures 1, 2, and 3 illustrate the symptoms of injury developing as a result of the feeding of individual leaf hoppers upon restricted areas of the leaf petioles of Ladino clover for 24 hours.

Figure 1 shows various stages in the wilting of the leaves. Some leaves were entirely wilted and shriveled at the end of the 24-hour feeding exposure, whereas others wilted slowly over a period of from 3 to 7 days. The constriction of the petioles which on this host

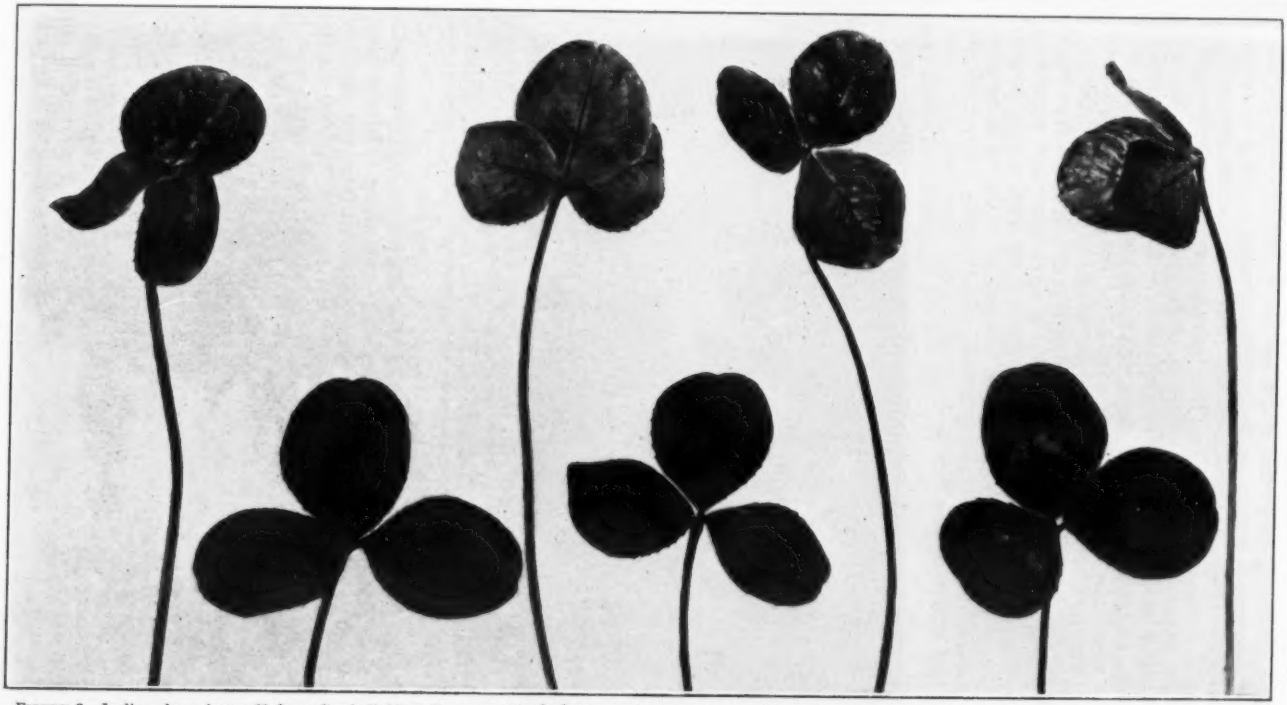

FIGURE 2.—Ladino clover leaves 20 days after individual *Empoasca fabae* had fed upon restricted areas of the petioles about 1 inch from the blades for 24 hours. The regions fed upon show no external signs of injury, but the petioles have reddened above these regions, and the leaflets are rigid, cupped, and a mottled red ,yellow ,and green color. The three lower leaves are uninjured checks from the same plants as the leaves above. Drawing by R. C. Steadman.

preceded the complete wilting of the leaf blades is evident at the point of insect injury. Figure 2 shows the symptoms that developed gradually on about 90 percent of the leaves which did not wilt as a result of the feeding injury. These leaves appeared perfectly normal for 2 or 3 days, then became yellowish or grayish green and began to redden from the margins inward from 6 to 8 days after the feeding exposure. Approximately 10 days after the reddening started, the leaflets were rigid, cupped, and a mottled red, yellow, and green color. The petioles also reddened back to the points of feeding, but in no case did symptoms develop below this point or upon other leaves upon the plants. Within a week or 10 days after the symptoms became intense, the colors faded somewhat and the leaf margins became necrotic and rolled either upward or downward as shown in figure 3. Once started, necrosis proceeded rapidly, and within a few days the entire leaf was dead and brown.

FIGURE 3.—Later stage in the reddening and yellowing of Ladino clover leaves caused by the feeding of individual *Empoasca fabae* upon restricted areas of the petioles for 24 hours. The colors have dulled somewhat and the leaflet margins are rolling and becoming necrotic. Photographed 29 days after the feeding exposure ended. Fifteen punctures were made with a fine glass needle into the petiole of the leaf at the right (injured region between ink marks), but no symptoms developed. The leaf at the left is an uninjured check.

Using the same technic for confining leaf hoppers to restricted areas of petioles, the writer prepared material and made microchemical tests for starch and reducing sugars on sections cut from the unwilted leaf blades at intervals after individual leaf hoppers had fed on the petioles for 24 hours. These tests revealed (1) that there was a gradual accumulation of reducing sugars and starch in the mesophyll cells of the leaf blades, first noticeable about 5 days after the feeding exposure ended; and (2) that the development and increase in intensity of the pathological symptoms were correlated with the gradual accumulation of carbohydrates in the leaves.

These facts suggested the hypothesis that the reddening and yellowing of legumes caused by this leaf hopper result from localized over-accumulation of the carbohydrate products of photosynthesis above the points where the injury to the phloem tissue of the vascular bundles has retarded or stopped the backward movement of sugars from the manufacturing cells. A survey of the literature dealing with the

relation of carbohydrate accumulation to the reddening and yellowing of leaves revealed that this hypothesis rested on a reasonably sound physiological basis. Further experimental work was planned, therefore, to test this hypothesis.

EXPERIMENTAL METHODS AND RESULTS

ARTIFICIAL PRODUCTION OF SYMPTOMS OF LEAF-HOPPER INJURY

It appeared that the production by artificial means of symptoms comparable with those caused by the feeding of the potato leaf hopper would be a critical test of the validity of the hypothesis that the insect acts merely as a mechanical agent and that localized over-accumulation of carbohydrates produces the pathological symptoms. Experiments were planned, therefore, in which attempts were made to injure the vascular bundles in petioles and stems by various mechanical means.

Glass-Needle Injury to Clover Petioles

The first attempt to produce by mechanical means the symptoms of *Empoasca fabae* injury to Ladino clover paralleled the first feeding test in which individual leaf hoppers were confined upon restricted areas of the leaf petioles for 24 hours. A duplicate series of comparable plants was used, and an attempt was made to simulate the mechanical injury caused by the leaf hopper in feeding by repeated puncturing of a restricted region of the leaf petioles with an extremely fine glass needle. Before attempting to injure the petioles with the glass needle, sections had been cut and the location of the five vascular bundles in the petiole determined by microscopic examination. Fifteen punctures were made in a region of the petiole approximately one-half inch in length, five attempts being made to puncture each of the three main bundles. The petioles of 28 leaves were injured in this way between November 12 and 18, 1930. The plants were kept in a greenhouse at 50° to 55° F. and observed daily until December 13. The results secured were negative, no pathologic symptoms developing on any of the injured leaves. Microscopic examination of the petioles in the region of injury showed that in all cases one or more of the bundles had been torn or injured to some extent, but in none had there been so complete a disruption and plugging of the phloem region as is observed frequently in bundles injured by *E. fabae*.

Partial Severing of Clover Petioles with Razor Blade

In order to obtain a complete stoppage of movement through certain bundles and leave others apparently unimpaired, a condition the writer believes to be comparable with that often resulting from the feeding of an adult *Empoasca fabae* upon a petiole, an attempt was made to sever part of the bundles in Ladino and red clover petioles by means of a safety-razor blade. Microscopic examination of petiole sections had indicated the possibility of severing 2 of the 5 bundles by cutting half way through from the flat side of the petiole and of severing 3 bundles by cutting half way through from the concave side.

On November 20, 1931, the petioles of 18 leaves of Ladino clover and 2 leaves each of Tennessee and Italian red clover were partially severed at a point about 1 inch from the leaf blade. In each case

half of the petioles were cut from the flat side and half from the concave side. The petioles were injured between 10 and 10:30 a.m. At this time the sun was shining brightly and the temperature in the greenhouse was between 80° and 85° F. The greenhouse was unshaded and the plants were in direct sunlight.

Since conditions seemed favorable for rapid transpiration, it appeared desirable to observe the injured leaves at frequent intervals during the remainder of the day with the hope of determining how much destruction of xylem in the petiole was necessary to cause wilting of the blade. The first observations were made at 11 a.m., one-half hour after the injuries were completed. At this time 5 of the 18 Ladino clover leaves and 1 of the 2 Tennessee red clover leaves

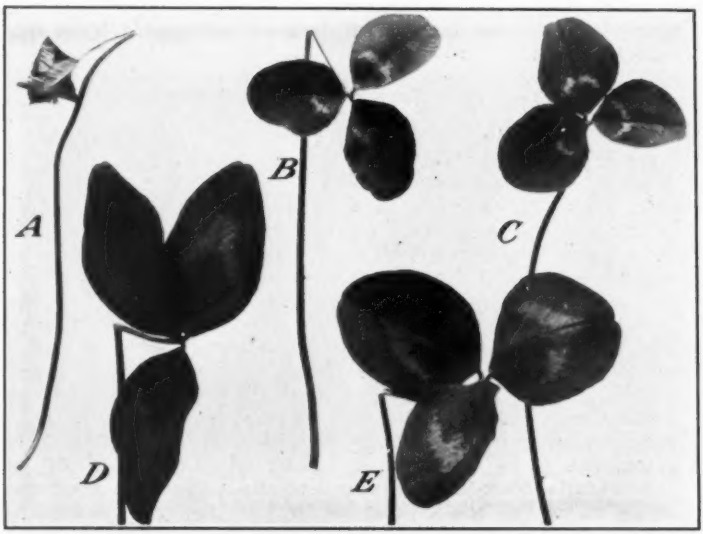

FIGURE 4.—Leaves of Ladino clover (*A*, *B*, and *C*) and of Italian (*D*) and Tennessee (*E*) red clovers 4 days after the petioles were partially severed by means of a safety-razor blade. The plants bearing these leaves had been in a greenhouse exposed to bright sunshine daily and at a temperature ranging from 75° to 85° F. The only leaf that wilted (*A*) had all 5 veins in the petiole severed as shown in figure 5. *C* is an uninjured check.

were flaccid. One and one-half hours later 1 Ladino leaf had regained its turgidity, but the other 5 leaves were still flaccid. At 2 p.m. the condition of the leaves appeared unchanged. At 3:30 p.m. a second Ladino leaf and the Tennessee red clover leaf had regained their turgidity, but the remaining 3 Ladino leaves were now definitely wilted and curling. At 5 p.m. the 3 Ladino leaves were still more wilted and curled, whereas all the remaining injured leaves appeared quite turgid in spite of the fact that several of the blades were hanging by only a slight attachment to the petiole at the point of the partial severing.

The condition of the leaves appeared unchanged the following morning, and no noticeable change occurred during that day. By the next day the three wilted leaves were shriveled and brown back to the point at which the petiole had been partially severed. The

other injured leaves were still turgid and a healthy green color. Four days after the petioles had been injured, a wilted and shriveled Ladino leaf, 3 partially severed but turgid leaves (1 Ladino clover and 1 Tennessee and 1 Italian red clover), and an uninjured Ladino leaf were removed and photographed (fig. 4). Freehand sections were then cut from each petiole at the point of injury and camera-lucida drawings (fig. 5) were made to show the amount of unsevered tissue connecting the blade with the petiole. They show that every bundle was severed in the petiole of the Ladino leaf that wilted and shriveled (fig. 4 and fig. 5, *A*), whereas only 1 bundle remained unsevered in the Ladino leaf that remained turgid in spite of severe petiolar injury (fig 4. and fig. 5, *B*). Two bundles remained unsevered in the petiole of the Italian red-clover leaf that remained turgid (fig. 4 and fig. 5,

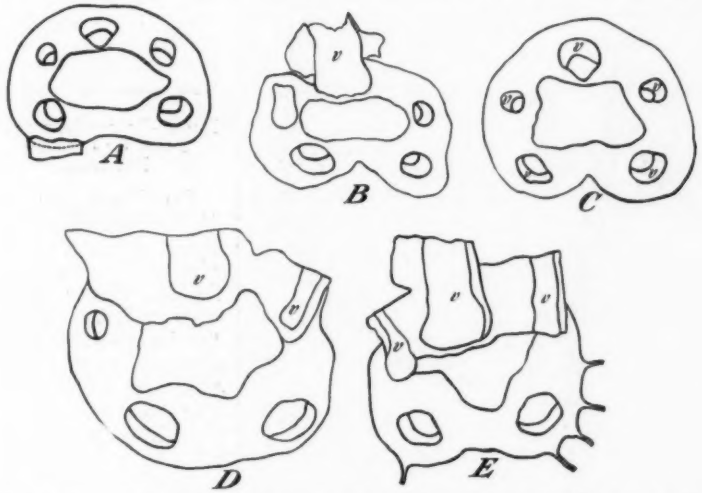

FIGURE 5.—Camera-lucida drawings of freehand sections cut across the petioles of the leaves illustrated in figure 4 at a point just below the partially severed region; *v*, the unsevered veins in each petiole. Note that the Ladino leaf that wilted (*A*) had all 5 veins severed, whereas the Ladino leaf that remained turgid (*B*) had only 1 unsevered vein in the petiole. The Italian red-clover leaf (*D*), with 2 unsevered veins, and the Tennessee red-clover leaf (*E*), with 3 unsevered veins, also remained turgid. Petiole section *C* is from the uninjured Ladino leaf.

D) and 3 remained unsevered in the petiole of the turgid Tennessee red clover leaf (fig. 4 and fig. 5, *E*).

This experiment shows that mechanically severing 3 or 4 of the 5 vascular bundles in a clover petiole will not cause a rapid wilting of the tissue above the injury, even under conditions favorable for transpiration. This condition sometimes occurs after an adult *Empoasca fabae* has fed for 24 hours upon a comparable region of the leaf petiole. It shows further that, as would be expected, complete stoppage of water movement to the leaf blade by mechanical severing of all the bundles in a petiole results in a rapid wilting similar to that caused by the leaf hopper.

The leaves with partially severed petioles (13 Ladino, 1 Tennessee red, and 1 Italian red) remaining after November 24 were kept in a warm greenhouse and observed daily. Twenty days after the petioles

were partially severed, one Ladino leaf began to redden, and 10 days later the entire blade was mottled red and yellowish green. The remaining leaves remained turgid and green until the experiment was ended.

During the course of the experiment just described it was observed that accidentally a Ladino clover leaf had been pulled almost loose from the stem and was hanging attached by a single vascular bundle. The leaflets of this leaf were cupping, reddening, and yellowing in a manner quite comparable to that occurring after an adult *Empoasca fabae* had been confined on the petiole, except that in the former case the reddening extended entirely back to the base of the petiole where food movement was interrupted. This suggested that the base of the petiole was a favorable place for mechanically severing part of the veins, and a further experiment was planned.

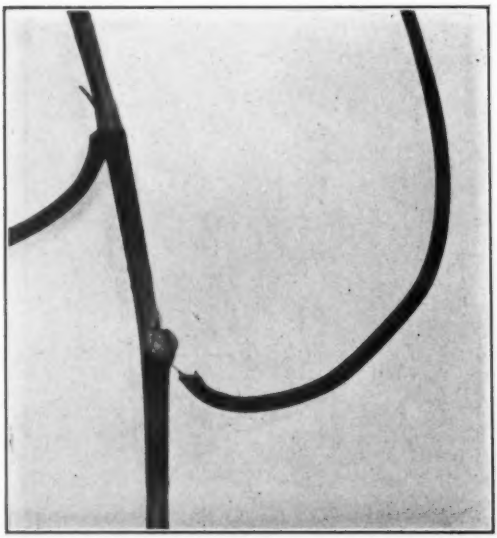

FIGURE 6.—Ladino clover stem showing upper petiole attached in normal manner and lower petiole attached by a single exposed vascular bundle. The latter condition was attained by pulling upon the petiole while cutting carefully at its base with a safety-razor blade. The stem tip bearing these leaves is shown in figure 7.

RAZOR-BLADE INJURY AT BASE OF CLOVER PETIOLES

On December 16, 1931, 4 leaves near the tip of a runner on a vigorously growing Ladino clover plant were pulled loose at the base of the petioles and part of the vascular bundles in the loosened leaf sheaths were severed with a razor blade. It was evident that 1 leaf remained attached to the stem by a single bundle, for this vein was exposed for approximately three-sixteenths of an inch (fig. 6). The exact amount of injury to the vascular elements of the other 3 petioles could not be definitely determined, although it was evident that not more than 1 or 2 vascular bundles remained unsevered in any case.

It was soon evident that 1 leaf had been too severely injured, for it became quite flaccid and slowly shriveled. The other 3 leaves also became slightly flaccid after the petioles were injured, but soon regained their turgidity, and after 3 days began to show faint signs of reddening. The symptoms intensified gradually, and by 8 days after the injury the blades of 2 of the leaves were streaked with red. Fifteen days after the petioles were injured the blades of these 2 leaves were a mottled red, yellow, and green, and their petioles were entirely reddened to the point of injury at their bases. The coloration be-

came more intense and the cupping of the leaflets became more pronounced during the next week. Twenty-three days after the petioles were injured the experiment was ended and a photograph taken to show the cupped discolored leaflets of the leaf attached by a single vascular bundle as compared with the green expanded leaflets of the normally attached leaves on the same stem tip (fig. 7).

This experiment shows that by mechanically severing all but one of the vascular bundles in a petiole symptoms can be produced that are almost identical with those caused by the feeding of a potato leaf hopper upon the petiole of a Ladino clover leaf. It would seem probable that in the former case the pathological symptoms were due to the accumulation of carbohydrates in the leaf. In order to study this question further it appeared desirable to have a rapid method of

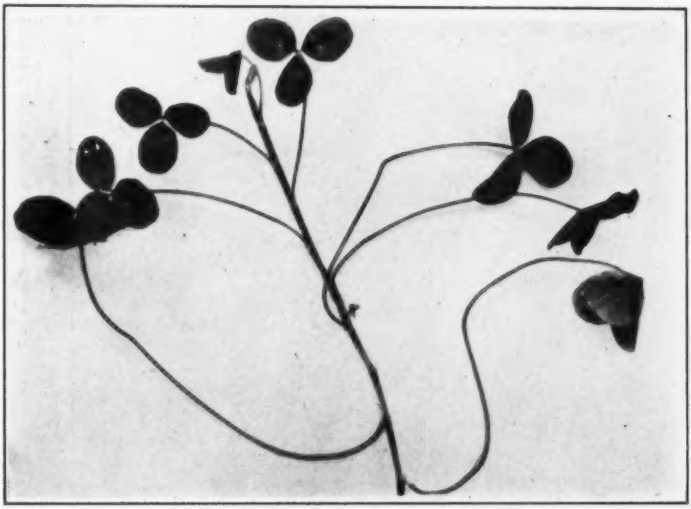

FIGURE 7.—Ladino clover stem tip showing the normally attached leaves green in color. In the leaf attached by a single vein (lower right) the leaflets were rigid, cupped, and a mottled red, yellow, and green color. Photographed 22 days after the petiole was injured at its base. Note the similarity of these symptoms to those caused by the feeding of a potato leaf hopper for 24 hours upon a restricted region of a Ladino clover petiole (fig. 2).

girdling clover petioles and alfalfa stems. The literature of plant physiology on translocation of food suggested the possibility of using live steam as a girdling agent.

GIRDLING ALFALFA STEMS AND CLOVER PETIOLES WITH LIVE STEAM

A preliminary experiment made December 31, 1931, showed that the living cells in a localized region could be killed by holding alfalfa stems and Ladino clover petioles for 15 to 30 seconds in a jet of live steam issuing under about 10 pounds pressure from a small petcock in a steam pipe used for greenhouse heating. The stems and petioles shriveled at the points of steaming (figs. 8 and 9), but water continued to pass upward through the shriveled regions, and the shoot tips and leaf blades remained turgid. Within 15 days after the petioles were steamed the 6 Ladino leaves were rigid, and the leaflets were cupped

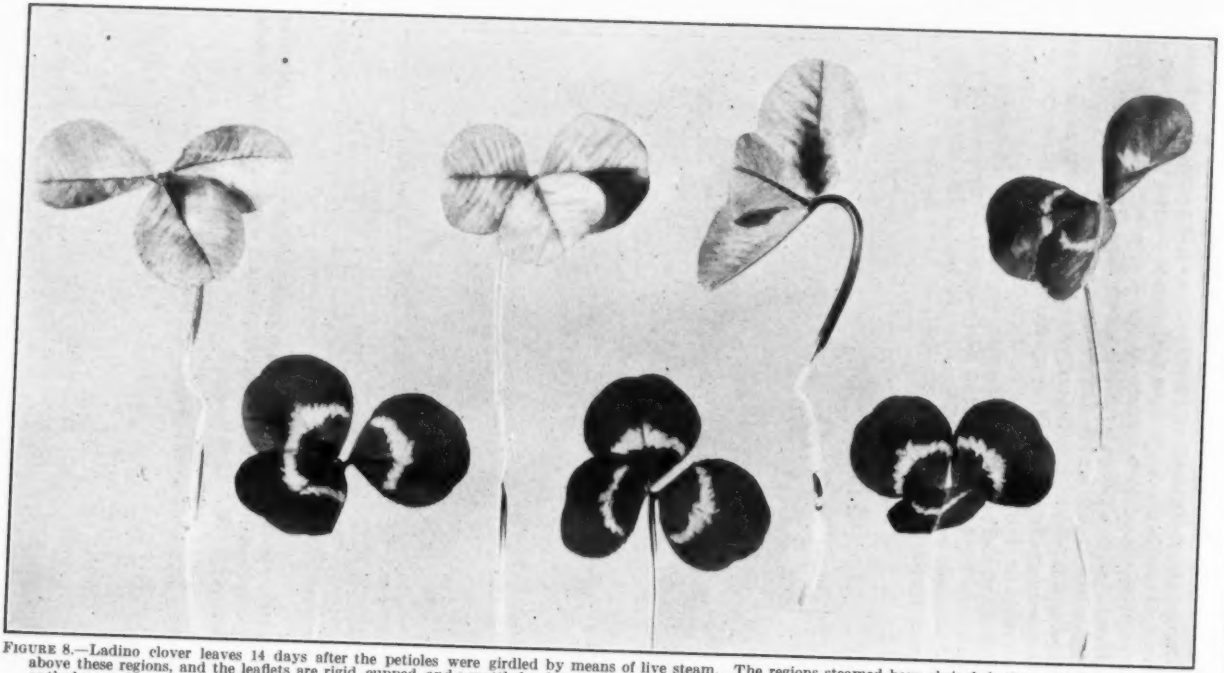

FIGURE 8.—Ladino clover leaves 14 days after the petioles were girdled by means of live steam. The regions steamed have shriveled, the petioles have reddened above these regions, and the leaflets are rigid, cupped, and a mottled red, yellow, and green color. The three lower leaves are uninjured checks from the same plants as the leaves above. The symptoms illustrated here are almost identical with those caused by the feeding of a potato leaf hopper upon a comparable region of a Ladino petiole for 24 hours (fig. 2).

and were mottled red, yellow, and green. The symptoms developed more slowly on the 6 alfalfa shoots, probably because of the greater amount of tissue above the girdles, which were made about 5 inches from the tips, and it was approximately 30 days after the stems were steamed before the entire shoot tips were yellowed and reddened.

This experiment was repeated on March 8, 1932, when 60 Ladino clover petioles and 77 Peruvian and Kansas alfalfa stems were steamed as described above. Within 4 days the Ladino petioles were reddening above the girdles and the leaf blades were beginning to redden from the margins inward, as in leaves injured by the leaf hopper. Ten days later, just 2 weeks after the petioles were steamed, every injured leaf had developed pathological symptoms, whereas the uninjured leaves appeared perfectly healthy and unchanged. Some of the injured leaves were already becoming necrotic, but most of them were rigid and the leaflets were cupped and mottled red, yellow, and green (fig. 8).

FIGURE 9.—Alfalfa stem showing shriveling of the region girdled by live steam and development of adventitious root primordia above the girdled region. Photographed 34 days after the stem had been held in a jet of live steam for 30 seconds. × 3.

From 4 to 6 days after the steaming the alfalfa stems began reddening or purpling above the girdles, and the green color of the leaves slowly faded to yellow-green. Yellowing then began between the veins of the leaflets and gradually spread until the entire shoot tips were yellowed and reddened. Figure 10 shows a yellowed and reddened alfalfa shoot tip 28 days after the living cells in a localized region of the stem were killed with live steam, and a healthy green ungirdled shoot from the same plant.

On March 12, 1932, 37 Ladino clover petioles and 40 alfalfa stems were steamed for approximately the same length of time as in the previous experiment. Symptoms similar to those just described developed on the leaves and shoot tips distal to the region steamed.

These experiments show that symptoms comparable with those caused by the feeding of the potato leaf hopper on clover petioles or alfalfa shoot tips can be produced by artificially girdling these structures by the use of live steam. In order to prove definitely that the reddening and yellowing of the girdled leaves and shoots were accompanied by carbohydrate accumulation, samples of these leaves and of healthy green leaves from the same plants were preserved in alcohol for analysis. The results of these analyses are presented in the following section and are compared with analyses of leaves reddened and yellowed by leaf-hopper infestation.

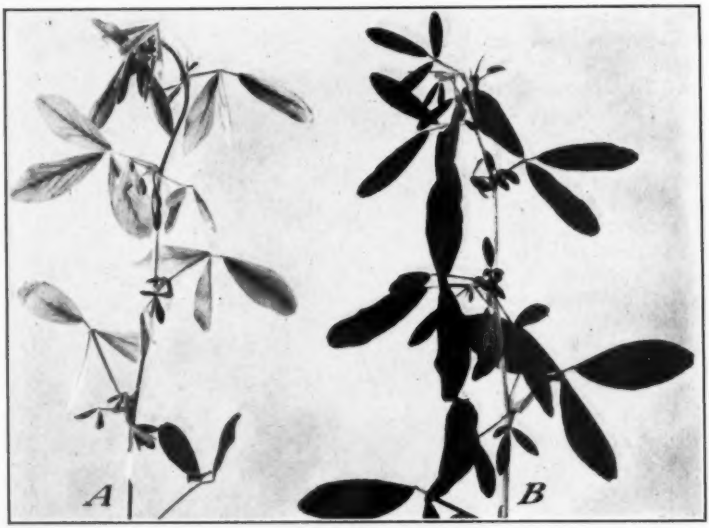

FIGURE 10.—Shoot of alfalfa (*A*) yellowed and reddened by girdling the stem with live steam, compared with a healthy green ungirdled shoot (*B*) from the same plant. Photographed 28 days after the stem of the shoot *A* had been held in a jet of live steam for 30 seconds. The symptoms here illustrated are practically identical with those caused by infesting caged alfalfa plants with potato leaf hoppers.

PHYSICAL AND CHEMICAL CHANGES IN ALFALFA AND CLOVER LEAVES AFTER LEAF-HOPPER INJURY

MOISTURE CONTENT

On September 1, 1931, yellowed and green shoots of Arizona Common alfalfa (F. C. no. 15837) were collected from rod-square field cages, one infested with *Empoasca* from potatoes and the other uninfested. The cut shoots were wrapped at once in moist towels, placed in a vasculum, and taken to the laboratory. The samples were taken between 10 a.m. and 2 p.m.; the day was clear and warm. In the laboratory, a handful of shoots was taken at random from the middle of each bundle, the leaves were at once picked off into tared weighing tubes, and the moisture content was determined in duplicate by oven-drying the material for 24 hours at 85° C. followed by 48 hours at 100°. The green weight of the samples used ranged from 10 to 16 g.

On August 29 and 30, 1932, yellowed and green alfalfa shoots were collected from the same pair of cages as in 1931 and from an additional

pair of similar cages nearby in the same alfalfa field. These samples were handled in exactly the same manner as those of the previous year.

The results of the moisture-content determinations are recorded in table 1 along with related data. They show that in both 1931 and 1932 the leaves of alfalfa yellowed by the leaf hopper had a higher dry-matter content than the green leaves. The increased dry-matter content of all the 1932 samples was probably due to the fact that they were taken in the middle of a severe drought.

TABLE 1.—*Chemical and physical data on alfalfa leaves yellowed by the potato leaf hopper (Empoasca fabae) as compared with normal alfalfa leaves, Arlington Experiment Farm, Rosslyn, Va., 1931–32*

Year	Condition of alfalfa leaves	Dry matter	Osmotic pressure of sap	Chemical composition on basis of green weight						
				Reducing sugar [1]	Sucrose [1]	Total sugar [1]	Starch	Total acid-hydrolyzable substances [1]	Soluble nitrogen	Insoluble nitrogen
		Percent	*Atmospheres*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1931	Yellowed	32.30	28.6	0.84	0.59	1.43	3.25	.....	0.64	1.09
				.71	.53	1.24	3.25	.....	.64	1.13
	Healthy green	19.71	16.9	.21	.23	.44	1.66	.....	.59	1.17
				.16	.28	.44	1.32	.....	.56	1.19
1932	Yellowed	36.84	26.1	1.51	1.00	2.51	3.47	8.39	[2] .163	.943
				1.33	.93	2.26	3.26	7.95	.191	.890
	Healthy green	32.75	22.1	.24	.60	.84	.49	3.00	.077	1.239
				.27	.59	.86	.45	3.01	.087	1.251
	Yellowed	37.08	28.5	1.02	.97	1.99	2.55	5.34	.263	.979
				1.13	1.00	2.13	.....	5.50	.281	.959
	Healthy green	36.12	.....	.26	.69	.95	.85	3.30	.143	1.328
				.24	.66	.90	.58	3.06	.147	1.294

[1] Expressed as dextrose.
[2] Chlorophyll nitrogen not included because of analytical method employed in 1932.

On September 10, 1931, two pairs of samples of reddened and green leaves of Ladino clover (F. C. no. 14048) were collected from cheesecloth cages, 2 by 3 feet, infested with *Empoasca* from cowpeas, and from similar uninfested cages. These samples were handled in the same way as the alfalfa except that only the leaves were brought into the laboratory. The samples were collected between 11 a.m. and 1 p.m.; the day was clear and warm. In the laboratory the procedure with these leaves was the same as with the alfalfa, except that only the upper inch of petiole, usually the only part of this structure that had reddened, was picked off with the reddened blades. The green weight of these samples ranged from 13 to 18 g.

On August 29, 1932, reddened and green leaves of Ladino clover were collected from infested and uninfested field cages and one pair of samples was prepared as just described.

The results of these moisture-content determinations are presented as percentage of dry matter in table 2. They show an increase in percentage of dry matter for the injured leaves similar to that in yellowed alfalfa leaves.

TABLE 2.—*Chemical and physical data on Ladino clover leaves reddened by the potato leaf hopper (Empoasca fabae) as compared with normal leaves, Arlington Experiment Farm, Rosslyn, Va., 1931–32*

Year	Condition of clover leaves	Dry matter	Osmotic pressure of sap	Chemical composition on basis of green weight					
				Reducing sugar [1]	Sucrose [1]	Total sugar [1]	Starch	Soluble nitrogen	Insoluble nitrogen
		Percent	*Atmospheres*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1931	Reddened	26.31	24.9	2.93	1.07	4.00	3.53	0.67	0.94
	Healthy green	20.99	15.8	.40	.27	.67	1.83	.42	1.24
	Reddened	27.79	35.1	1.75	.78	2.53	2.26	.67	1.00
	Healthy green	19.89	13.5	.39	.38	.77	.77	.45	1.22
	Reddened [2]			2.35	1.04	3.39		.19	.67
	Healthy green [3]			.15	1.24	1.39		.12	1.04
1932	Reddened	31.26		2.32	- 1.09	3.41	1.67	[4] .149	.675
	Healthy green	25.59		.40	.45	.85	.54	.085	.905

[1] Expressed as dextrose.
[2] Plants were grown in the greenhouse; individual leaf hoppers were allowed to feed upon restricted areas of the petioles for 8 or 16 hours.
[3] Plants were grown in the greenhouse and were kept free from leaf hoppers.
[4] Chlorophyll nitrogen not included because of analytical method employed in 1932.

OSMOTIC PRESSURE OF SAP

After the samples for the moisture-content determinations had been placed in weighing tubes, similar 50-g samples were weighed out. Each sample was transferred to a Pyrex test tube 1¼ by 12 inches; the tube was then tightly stoppered and immediately plunged into a freezing mixture of ice and calcium chloride. The material was kept frozen until the following morning, when the tubes were removed from the freezing mixture and the samples thawed out at room temperature. In 1931 the sap was pressed out by means of a heavy steel cylinder and piston in a Lentz hydraulic press and collected in a 50-cc Florence flask. The pressure employed was gradually increased to approximately 20,000 pounds to the square inch. In 1932 a lighter cylinder and piston were used in a Carver laboratory press and the final pressure obtained was 10,000 pounds to the square inch.

After allowing the press to drain for 5 minutes in all cases, the sample of expressed sap was shaken, 15 ml was poured into a Pyrex test tube, and the freezing-point depression was determined by the use of a Heidenhain thermometer. A mixture of ice and table salt was used in the cryoscope, and all determinations were made with the temperature of the freezing mixture ranging between −10° and −18° C. Triplicate readings of undercooling and freezing point were always made, and the average of the three readings was used in making the calculations. The zero point of the Heidenhain thermometer was checked with distilled water before each set of determinations, and the readings were properly corrected. By means of a correction factor secured from the table of Harris (*11*), the observed freezing-point depression was corrected for the amount of undercooling. The corrected freezing-point depression was converted into atmospheres of osmotic pressure from the table of Harris and Gortner (*12*).

The results secured, expressed as atmospheres of osmotic pressure, are presented in tables 1 and 2 along with related data. They show that in 1931 the leaves of alfalfa yellowed by *Empoasca* had a much higher osmotic pressure than the green leaves, 28.6 atmospheres as compared with 16.9 atmospheres. A similar difference was found to exist between Ladino clover leaves reddened by *Empoasca* and green Ladino clover leaves: 24.9 atmospheres as compared with 15.8 atmospheres in one set of samples, and 35.1 as compared with 13.5 in the other. In 1932, the differences obtained with alfalfa were in the same direction but considerably less in magnitude than those of 1931 because of the increased osmotic pressure of the juice from the green leaves. The juice from the samples of yellowed leaves showed osmotic pressures of 26.1 and 28.5 atmospheres and the juice from the sample of green leaves showed an osmotic pressure of 22.1 atmospheres. This rise in osmotic pressure is correlated with the increased dry-matter content of the green leaves, caused doubtless, as pointed out above, by the dry weather prevailing at the time of sampling. No Ladino clover samples were available for cryoscopic determinations in 1932.

Chemical Composition, 1931

After the samples for the cryoscopic determinations had been placed in the freezing mixture, duplicate 50-g samples similar to those used in the moisture-content and cryoscopic determinations were weighed out. Immediately after being weighed each sample was transferred to a quart glass jar containing 400 ml of hot 95-percent alcohol and 0.25 g of calcium carbonate. These jars were lightly sealed, then placed in a water bath, and the alcohol was boiled for 15 or 20 minutes to stop enzyme action. At the end of this period, the jars were tightly sealed, lifted from the water bath, and after being cooled were stored in a pasteboard box for approximately 2 months before the analyses were made.

The sugars present in the samples were extracted by means of the preserving alcohol as percolate, and the amount of reducing sugars and total sugar present in the extracts was determined by following the procedure given by the Association of Official Agricultural Chemists (*1*) for the determination of sugars in feeding stuffs. The weight of the cuprous oxide precipitates was obtained by direct weighing, and their equivalents in dextrose were obtained from Munson and Walker's table (*32, pp. 139–146*). The nitrogen present in the extracts was determined by the unmodified Kjeldahl method of the Association of Official Agricultural Chemists.

The residues from the alcoholic extraction were oven-dried, weighed, and finely ground in a small hand mill. The starch content of the residues was determined by the Walton and Coe method (*33*) for the determination of starch in the presence of interfering polysaccharides, except that the picric acid colorimetric modification, described by Coe and Bidwell (*4*), was used to determine the amount of dextrose present after acid hydrolysis. The nitrogen present in the residues was determined by the unmodified Kjeldahl method.

The results of the analyses, expressed as percentages of green weight of the leaves, are presented in tables 1 and 2. They show that in 1931 the leaves of alfalfa yellowed by *Empoasca* were higher in reducing sugar, sucrose, total sugar, starch, and alcohol-soluble

nitrogen but lower in insoluble nitrogen than the healthy green leaves from the uninfested cage. Similar differences were found to exist between leaves reddened by *Empoasca* and healthy green leaves of Ladino clover. Both sets of analyses showed the reddened leaves to contain more carbohydrates and soluble nitrogen but less insoluble nitrogen than the green leaves.

The results of a third set of analyses of leaves reddened by *Empoasca* and healthy green leaves of Ladino clover are also presented in table 2. The reddened leaves for this set of analyses were secured by allowing individual *E. fabae* confined in small insect cages to feed for 8 or 16 hours upon restricted areas of the leaf petioles about 1 inch from the blades. From February 17 to February 28, 1931, a total of 104 leaves distributed on 17 different plants had the petioles exposed in this way to injury by *E. fabae*. By March 14 many of the leaves showed advanced symptoms of the injury (fig. 2). On this date 46 reddened and 46 healthy green leaves were collected and preserved in alcohol for chemical analysis. These samples were collected between 10 and 11 a.m.; the day was clear and warm.

In collecting the leaves, the petiole was first severed near its base and then cut through again just above the ink mark indicating the upper limit of the small cage in which the hopper had been confined during the feeding exposure. The blade and short section of reddened petiole were then placed in a low-form Stender dish to prevent loss of moisture. Immediately after the reddened leaves had been removed from a plant, an equal number of healthy green leaves were removed in the same way and placed at once in another Stender dish. The net weight of each sample was approximately 6 g. After being preserved in alcohol, the samples were stored in a dark cupboard for about 1 month, until the analyses were made.

The results of these analyses show that the Ladino leaves reddened by *Empoasca* contained more reducing sugar, total sugar, and soluble nitrogen but less sucrose and insoluble nitrogen than healthy green leaves from the same plants. These results are in general accord with the others presented in this table which are based upon 50-g samples produced in infested and uninfested cages in the field during the summer months.

Because of the small size of the samples produced in the greenhouse, no starch determinations could be made. However, sections cut from a reddened leaf and stained with iodine-potassium iodide showed the chloroplasts to be gorged with starch grains, whereas a similar test made on sections from a green leaf showed the chloroplasts to be practically free from starch except those in the guard cells of the stomata in the lower epidermis.

Chemical Composition, 1932

On August 29 and 30, 1932, when samples of alfalfa and Ladino clover leaves were taken from infested and uninfested field cages for moisture-content and cryoscopic determinations, duplicate 50-g samples similar to those of 1931 were preserved in alcohol for analysis. These samples were stored for approximately 2 months, then extracted and analyzed for carbohydrates and nitrogen by methods comparable in general with those used the previous year. However, in determining sugars in 1932 the reduced copper was determined by the perman-

ganate method (volumetric) instead of by direct weighing. Further, starch was hydrolyzed by fresh saliva instead of by freshly prepared malt diastase, and in addition to the starch determinations reported as starch 0.2-g samples of the oven-dry residues from alcoholic extraction were subjected to direct acid hydrolysis. The results of these determinations are reported as dextrose under the heading Total acid-hydrolyzable substances. In 1932 the Kjeldahl determinations were modified to include the nitrogen of nitrates (Gunning method). Further, the chlorophyll nitrogen was not included with the other soluble nitrogen; since after the alcoholic extracts were made up to volume, the chlorophyll was separated out in a layer of chloroform at the bottom of the volumetric flasks and subsequently discarded except where it was necessary in a few cases to determine total solids in the extracts. It is thought that this small amount of nitrogen lacking from the analyses of both the yellowed and green leaves is of little consequence as far as the significance of the results is concerned. The results of the 1932 analyses are also presented in tables 1 and 2.

The data in table 1 show that in 1932, as in 1931, the yellowed leaves of alfalfa from field cages infested with *Empoasca* were higher in reducing sugar, sucrose, total sugar, starch and soluble nitrogen but lower in insoluble nitrogen than the healthy green leaves. They show further that the yellowed leaves were much higher in total acid-hydrolyzable substances than the green leaves from uninfested cages. The data presented in table 2 show that the chemical changes in leaves of Ladino clover reddened by *Empoasca* in 1932 were similar in all respects to those in 1931 and in yellowed alfalfa leaves both years. The determinations of total acid-hyrolyzable substances in the 1932 Ladino samples, not presented in the table, showed that the reddened leaves contained 4.09 percent, whereas the green leaves contained only 2.30 percent. This change is comparable with the change occurring in yellowed alfalfa leaves (table 1).

PHYSICAL AND CHEMICAL CHANGES IN ALFALFA AND CLOVER LEAVES AFTER INJURY BY LIVE STEAM

On March 23, 1932, a sample of Ladino clover leaves, reddened and yellowed by girdling the petioles with live steam, was preserved in alcohol for analysis. This sample consisted of 25 leaves, the petioles of which had been steamed on March 8, and 29 leaves, the petioles of which had been steamed on March 12, 1932. A comparable sample of healthy green leaves from the same plants was preserved at the same time. The leaves were collected between 1 and 1:30 p.m.; the day was clear and warm. The sampling technic was similar in all respects to that just described for the sample of Ladino clover leaves reddened by the feeding of individual *Empoasca fabae* upon restricted areas of the leaf petioles. The green weight of the 54 yellowed and reddened leaves was 12.686 g; that of the same number of green leaves was 13.585 g.

On April 16, 1932, samples of yellowed and healthy green alfalfa leaves, comparable with those just discussed, were preserved in alcohol. The stems of the alfalfa shoots bearing the yellowed leaves had been girdled with live steam on March 8 and 12, 1932. The sample of yellowed leaves was taken from 70 girdled shoots distributed on 26 plants. The sample of green leaves was collected from the same number of ungirdled shoots on the same plants. The green

weight of the sample of yellowed and reddened leaves from the steamed shoots was 90.907 g; that of the green leaves from unsteamed shoots, 92.7 g. These samples were kept in a dark place for approximately 6 months and were analyzed for carbohydrates and nitrogen at the same time as the samples from infested and uninfested field cages preserved on August 29 and 30, 1932. The methods of analysis employed have already been outlined.

The results of the analyses of these samples are presented in table 3, and show that leaves of alfalfa and Ladino clover, yellowed and reddened by girdling the stems and petioles, respectively, with live steam, were higher in reducing sugar, sucrose, total sugar, total acid-hydrolyzable substances, and soluble nitrogen, but lower in insoluble nitrogen than healthy green leaves from the same plants. It shows further that the yellowed leaves of alfalfa were higher in starch than the green leaves from ungirdled shoots. Because of the small size of the Ladino samples, no starch determinations were

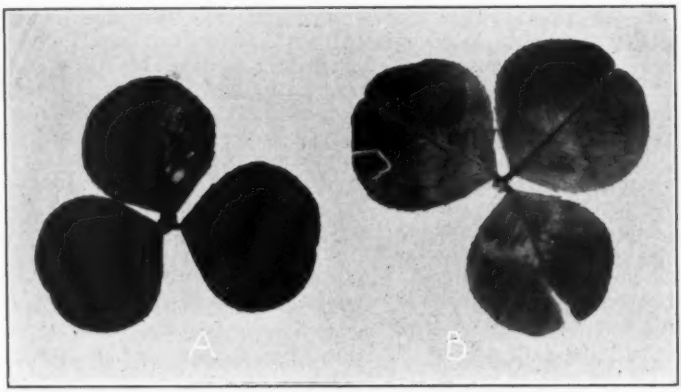

FIGURE 11.—Iodine test for starch in leaves of Ladino clover: *A*, Leaf reddened by girdling the petiole with live steam; *B*, a green ungirdled leaf from the same plant. The dark purple color of the reddened leaf indicates the starch congestion that occurred in the leaves with girdled petioles.

possible. However, figure 11, which shows a reddened and a green leaf stained with iodine-potassium iodide after removal of the pigments with boiling alcohol, illustrates the starch congestion which occurred in the leaves with girdled petioles.

These analyses indicate that the yellowing and reddening of the alfalfa leaves on girdled shoots and of the Ladino clover leaves with girdled petioles are due to an overaccumulation of carbohydrates above the girdled regions. The chemical changes occurring in alfalfa and Ladino clover leaves yellowed and reddened by the potato leaf hopper, as shown by the analyses presented in tables 1 and 2, are comparable in all respects with those occurring in the leaves with similar pathological symptoms produced by artificial girdling of petioles and stems by means of live steam. This would appear to be further evidence that this leaf hopper acts merely as a mechanical agent in producing the yellowing and reddening of forage legumes.

TABLE 3.—*Chemical data on alfalfa and Ladino clover leaves yellowed and reddened by artificial girdling of the stems and petioles, respectively, with live steam, as compared with the normal leaves, in greenhouse at the Arlington Experiment Farm, Rosslyn, Va., 1932*

Plant and condition	Green weight of samples	Dry matter	Chemical composition on basis of green weight						
			Reducing sugar [1]	Sucrose [1]	Total sugar [1]	Starch	Total acid-hydrolyzable substances [1]	Soluble nitrogen [2]	Insoluble nitrogen
Alfalfa: [3]	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Yellowed by girdling	90.907	32.46	1.15	0.95	2.10	8.78	13.52	0.095	0.504
Healthy green	92.700	26.46	.23	.57	.80	2.39	5.00	.084	1.057
Ladino: [4]									
Reddened by girdling	12.686	21.11	2.51	1.59	4.10	.....	4.42	.241	.456
Healthy green	13.585	16.17	.57	.65	1.22	.....	2.54	.066	.677

[1] Expressed as dextrose.
[2] Chlorophyll nitrogen was not included because of the analytical method employed in 1932.
[3] Samples were preserved in alcohol on Apr. 16, 1932 (35 to 39 days after the stems were steamed).
[4] Samples were preserved in alcohol on Mar. 23 (11 to 15 days after the petioles were steamed).

FURTHER EVIDENCE AGAINST VIRUS AND TOXIN HYPOTHESES

It appeared from the literature that no attempt had been made to produce symptoms characteristic of leaf-hopper injury by artificial inoculation of forage legumes or to determine whether the symptoms would develop on plants started as shoot cuttings from plants yellowed and reddened by leaf-hopper infestation. It seemed desirable, therefore, to make experiments along these lines to test further the possibility of a virus or toxin being present.

During the summer of 1931 three sets of artificial inoculations were made by means of (1) juice extracted from crushed leaves of yellowed alfalfa and from reddened Ladino clover plants from infested field cages, and (2) juice extracted from macerated adults and nymphs of *Empoasca fabae* removed from field cages in which they had fed and had produced typical symptoms of the injury on alfalfa and Ladino clover. In each set of inoculations, check plants were inoculated with juice from crushed green leaves from uninfested field cages and juice from macerated adults and nymphs of *Aceratagallia sanguinolenta* (Prov.), a leaf hopper occurring on alfalfa but not able to produce a yellowing of the leaves as does *E. fabae*. All inoculations were made by holding a drop of the extract in a flamed loop needle successively against the leaf blade, petiole, and stem of a healthy plant (alfalfa, Ladino clover, red clover, or cowpea) and scratching or pricking the plant with a flamed pointed needle through the drop of extract. An attempt was thus made to introduce the extract prepared from the macerated material into the healthy plant at from 10 to 20 different points. The inoculations were made and the plants held in an insect-proof greenhouse and observed at regular intervals over a period ranging from 10 to 14 weeks. Only negative results were obtained; and although the number of plants inoculated is not very large (82 in all), it appears that if a specific inciting agent (virus or toxin) is involved in the production of the symptoms of *Empoasca fabae* injury on forage legumes it is not readily transmissible by mechanical means.

In July 1932, 369 shoot cuttings were made from heavily infested and yellowed alfalfa plants growing in the field at the Arlington Experiment Farm. The cuttings were placed in flats of moist sand, fumigated with volatile nicotine, and taken into a screened greenhouse. At the same time, 80 shoot cuttings were made from infested reddened Ladino clover plants and were handled in the same way. Seventy-seven of the alfalfa cuttings and 79 of the Ladino cuttings rooted in the sand and developed new top growth which was healthy green in color. In August these rooted cuttings were placed in pots of soil, and the potted plants were kept under observation for approximately 5 months. No symptoms of the leaf-hopper injury developed on any of these plants. The negative results secured in these tests are further evidence that the symptom complexes caused by the potato leaf hopper on forage legumes are not due to the transmission of a virus or toxic substance by this insect.

Still further evidence on this point is furnished by data on the relation between length of feeding of individual *Empoasca fabae* upon restricted areas of the petioles of Ladino clover and the production of wilting or reddening and yellowing above the point of insect feeding. Were a virus or toxic substance introduced by the leaf hopper in feeding, it would seem probable, because of the potency of minute quantities of these substances, that a feeding exposure of 8 hours would produce almost as many and as severely injured leaves as a feeding exposure 2 or 3 times this length. Such evidently is not the case, since the data presented in table 4 show that a definite correlation exists between the length of time that individual *Empoasca fabae* feed upon leaf petioles of Ladino clover and the number of leaves that develop symptoms of the injury.

TABLE 4.—*Relation between length of feeding of individual Empoasca fabae upon restricted areas of the leaf petioles of Ladino clover and the production of symptoms of injury above the regions fed upon, in greenhouses at the Arlington Experiment Farm, Rosslyn, Va., between Jan. 8 and Mar. 24, 1931*

Feeding exposure (hours)	*E. fabae* used in test	Petioles fed on	Leaves wilted	Leaves reddened and yellowed	Leaves showing no symptoms
	Number	*Number*	*Percent*	*Percent*	*Percent*
8	23	49	0	18.4	81.6
16	23	56	0	62.5	37.5
24	17	38	44.7	47.4	7.9

Smith and Poos (*29*) found that a single leaf hopper in 24 hours of feeding upon a cowpea petiole made 14 punctures into the phloem and injured 5 of the 7 vascular bundles. It appears quite probable, therefore, that in 24 hours of feeding upon Ladino petioles, which contain only 5 vascular bundles, an adult *Empoasca fabae* would injure a high percentage of the petioles fed upon to such an extent that pathological symptoms (wilting or reddening and yellowing) would result. In 16 hours of feeding fewer petioles would be injured to this extent, and in 8 hours of feeding still fewer would have the phloem injured sufficiently to cause symptoms to develop.

It is recognized that the technic employed in these feeding tests is open to the criticism that the small cages made it necessary for the leaf hoppers to circle the petioles in seeking food and that perhaps under

natural conditions they would not feed in this manner. However, field observations of the habits of this leaf hopper reveal that in feeding it moves sidewise around the petioles and stems with great ease. It would appear that this habit of moving around the petioles and stems while feeding upon the veins is correlated with the ability of the potato leaf hopper to injure sufficient vascular tissue in petioles and stems to cause pathological symptoms to develop.

DISCUSSION

Evidence has been presented indicating that the reddening and yellowing produced on forage legumes by the potato leaf hopper are due, not to the injection of a specific inciting agent but to an accumulation of the carbohydrate products of photosynthesis above the points where a plugging of the phloem tissues is caused by the leaf hopper in its feeding upon the veins of stem tips, petioles, and leaf blades. A more detailed exposition of the writer's conception of the altered physiology underlying the production of the pathological symptoms on infested plants is here presented, together with a review of some of the literature bearing upon the various symptoms discussed.

The reddening and purpling of the leaves and stem tips appear to be due to the development of anthocyanin as a direct result of the overaccumulation of sugars in the regions distal to the points of feeding.

Boodle (*3*) in 1903 observed the development of anthocyanin in localized areas of the leaves of *Rheum* supplied by veins which had been accidentally severed. He obtained similar results with evening primrose, *Oenothera biennis* L., by severing the midrib of the leaf about its middle. He concluded that—

> this operation interrupted the channel by which the carbohydrate products of assimilation are normally removed from the leaf so that sugar accumulated above the cut and this concentration of soluble carbohydrates led to the development of anthocyanin.

Mirande (*18*) in 1907 reported that the reddening of the leaves of cherry and other plants associated with the lesions caused by the feeding of the larvae of several dipterous and lepidopterous insects was quite localized, anthocyanin forming only in the regions where local interruption of the phloem tissue led to the accumulation of glucose and tannins. He concluded that in the cases he studied the conditions of reddening were entirely chemical on the side of the plant and purely mechanical on the side of the insect. In the writer's opinion the same conclusion is warranted in the case of the reddening caused by the potato leaf hopper on forage legumes.

The yellowing of the leaves appears to be due to a diminution in the plasma and chlorophyll content of the chloroplasts in the mesophyll cells distal to the points of insect injury.

Molisch (*19*) in 1916 confirmed the observation of Zacharias (*34*) that the chloroplasts are the parts of the leaf cell richest in protein, and reported further that green leaves gave intense and yellow leaves very weak color reactions with the xanthoproteic, biuret, and Millons' tests because of the disappearance of the stroma of the chloroplasts during yellowing. Meyer (*17*) in 1918 showed that with age the plastids in the leaves of *Tropaeolum majus* L. become smaller and the chlorophyll content decreases. He correlated these differences with a breaking down of the reserve proteins in the plastids as the

leaves age. Kraus and Kraybill (*15, p. 71*) have observed in tomato stems and Reid (*22, 23, 24*) has observed in the leaves of squash seedlings a diminution in the plasma and chlorophyll content of chloroplasts similar to that attributed by Meyer to aging processes but brought about relatively early in the life of the plant by environmental conditions which produce an accumulation of carbohydrates in connection with a very limited nitrogen supply. The writer would consider the yellowing caused by the potato leaf hopper as a premature aging of localized areas of plant tissue brought about by an overaccumulation of carbohydrates and a relative or perhaps actual deficiency of nitrogen in the cells above the points where the hoppers have fed upon the veins. The shift of nitrogen in the yellowed and reddened leaves from the insoluble to the soluble form (tables 1, 2, and 3) would appear to indicate a breaking down of the reserve proteids in the plastids similar to that observed by Molisch and Meyer in leaves yellowing because of normal aging processes.

The rigidity of the reddened and yellowed leaves, which when separated from the plant remain turgid much longer than the healthy green leaves, appears to be due to the fact that these are high-sugar leaves and have an increased osmotic pressure.

The stunting of the plants infested with *Empoasca* appears to be due to their inability to translocate the manufactured carbohydrates from the leaves to the stem tips, where vegetative extension normally occurs. Kraus and Kraybill (*15*) have pointed out that retarded growth and starch accumulation would be the expected results if the manufactured carbohydrates in a plant were not utilized in vegetative extension or the production of reproductive parts.

The congestion of sugars and starch in localized regions of the injured plants apparently stimulates to development lateral buds which would remain dormant under normal conditions. The proliferation of lateral shoots is a frequent symptom of *Empoasca fabae* injury on alfalfa. This symptom may be considered an attempt on the part of the plant to restore its metabolic balance by utilizing in vegetative extension the rapidly accumulating carbohydrates.

Tip and marginal burning on the leaves and the wilting of entire leaves and shoot tips are symptoms which apparently may be due to different underlying causes on different hosts. The relatively late marginal necrosis which develops on mature clover and alfalfa leaves appears to be a final stage in the pathological symptoms caused by overaccumulation of carbohydrates in the leaves. On the other hand, the rather rapid burning of the leaf margins of infested potatoes and glabrous soybeans and the rapid wilting of entire leaves and shoots of seedling clover and alfalfa indicate that a serious interference with water movement in the plants must be involved.

By their histological studies Smith and Poos (*29*) showed that in some instances the feeding of an adult *Empoasca fabae* upon a petiole resulted in a plugging of part of the water-conducting tubes of the xylem, and they reported that the wilting of the plant tissue above the point of feeding is evidently a result of this vascular occlusion. However, the writer has already described an experiment which shows that mechanically severing 3 or 4 of the 5 vascular bundles in a clover petiole will not cause rapid wilting of the tissue above the injury, as occurs in some cases following the feeding of an adult *E. fabae* for 24 hours upon a comparable area of the petiole.

It appears from this experiment that the wilting must be due to a practically complete stoppage of water movement through the petiole or young shoot and that other factors in addition to partial vascular occlusion probably are involved.

Mention has been made of the theory advanced by Lutman (*16*) that the leaf hopper in sucking the juice from the veins removes the water from the tubes and from the adjacent sieve tubes, companion cells, and elongated parenchyma to such a degree that these structures plasmolyze and collapse to a point where they cannot regain their turgidity, and that as a consequence they die and become brown. Fenton and Hartzell (*6*) recorded distortion and collapse of the veins for a short distance beyond the immediate yellowed and necrotic area as a constant symptom of hopperburn on potato. The writer has observed a similar collapse of the veins just behind the injured area on the leaves of glabrous soybeans heavily infested with *Empoasca*. The marginal yellowing and browning on the leaves of these plants closely resembles the hopperburn of potato. The writer has called attention (fig. 1) to the collapse of the region of the petioles of Ladino clover upon which individual *E. fabae* have been confined for 24 hours. As shown in figure 1, the collapse of the petiole on this host precedes the complete wilting and shriveling of the blade and petiole above the point of feeding. It would appear from these observations that in addition to occlusion of a portion of the water-conducting tubes, plasmolysis and collapse of the living cells of the veins fed upon, and possibly the entrance of air into the disrupted bundles are factors in producing a stoppage of water movement to the living cells beyond the points at which the leaf hoppers have fed. The pathological symptoms resulting are (1) the wilting and browning of the entire leaf or young shoot if the leaf hoppers feed upon the petioles or young stems and (2) the drying out and browning of the leaf tip and margin if the leaf hoppers feed upon the veins on the under surface of the leaf blades.

Attention has been called to the similarity of the symptoms of this localized injury on forage legumes to those of the yellows diseases of plants which are systemic in nature and caused by viruses transmitted by leaf hoppers. It is interesting to note further that the chemical changes occurring in the leaves of alfalfa and Ladino clover, yellowed and reddened by *Empoasca fabae* and by artificial girdling of the stems and petioles with live steam, are similar in practically all respects to those reported by Spaeth and Kraybill (*31*) for the false-blossom disease of cranberry and by Rosa (*25*) and Shapovalov and Jones (*26*) for tomato yellows. It would appear, therefore, that overaccumulation of the carbohydrate products of photosynthesis may play a relatively important part in the production of the external symptoms characterizing some of the yellows diseases of plants as well as in the production of the symptoms of the localized injury caused by the potato leaf hopper on forage legumes.

SUMMARY

All available evidence indicates that the pathological symptoms caused by the potato leaf hopper on forage legumes are not due to the transmission of a virus by this insect.

The injection of some toxic substance into the plant during the feeding of the leaf hopper remains a possibility but is believed to be improbable.

Reddening and yellowing almost identical with those caused by the feeding of this leaf hopper can be produced artificially by mechanically severing all but one of the vascular bundles in Ladino clover petioles and by girdling Ladino clover petioles and alfalfa stems by means of live steam.

As compared with green leaves from uninfested plants, the yellowed and reddened leaves of alfalfa and Ladino clover are higher in dry matter, osmotic pressure of cell sap, reducing sugar, sucrose, starch, total acid-hydrolyzable substances, and alcohol-soluble nitrogen, but are lower in total nitrogen because of a marked decrease in the insoluble fraction.

The chemical changes in leaves reddened and yellowed by artificial girdling and by the potato leaf hopper are essentially the same.

It appears that the reddening and yellowing caused by this leaf hopper on forage legumes are due to an overaccumulation of the carbohydrate products of photosynthesis above the points where interruption of the functions of vascular tissues is caused by the leaf hopper in its feeding upon the veins of stem tips, petioles, and leaf blades.

A detailed discussion is given of the physiological changes underlying the various pathological symptoms.

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PARASITISM OF RHIZOCTONIA SOLANI ON SUGAR BEET [1]

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INTRODUCTION

The designation *Rhizoctonia solani* Kühn has commonly been used for the group of pathogenes causing cortical necroses and other disease manifestations of various host plants. Probably the best known disease of this type is the black scurf or rhizoctonia disease of potato (*Solanum tuberosum* L.). A serious root rot of sugar beets (*Beta vulgaris* L.) attributed to *R. solani* is also well known.

Considering the wide-spread distribution of the potato disease, the occurrence of root rot of sugar beets in fields previously cropped to potatoes suggests the possibility that the sugar-beet root disease is caused by organisms introduced with the potato crop, and thus the disease situation is intensified by the type of crop rotation. The problem is of especial interest in the Red River Valley section of Minnesota and North Dakota, since sugar beets commonly follow potatoes in cropping systems. The major purpose of this investigation was to determine if such interrelation existed between the diseases of the two hosts.

The existence of distinct strains within the *Rhizoctonia solani* group has been shown by many writers, notably Rosenbaum and Shapovalov (*20*),[3] Edson and Shapovalov (*4*), Matsumoto (*12*), Gratz (*8*), Van der Meer (*13*), Walker (*23*), Lauritzen (*11*), Wiant (*26*), and Elmer (*5*). In some cases strain differentiation has been based on morphological differences, but usually the criterion has been differences in pathogenicity or in growth form in artificial culture. Opinion has not been concordant as to the nature of these strains. Peltier (*17*), who carried on extensive comparative studies, concluded that "no marked specialization was noted in any of the strains", and the conclusions drawn by some other workers (*1*, *14*) coincide with this viewpoint.

Since it has been shown that the organisms included under the name *Rhizoctonia solani* are in fact a complex group of strains, any attack that might adequately test the possibilities of the problem needs to deal with many cultures collected over a considerable geographic area

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[3] Reference is made by number (italic) to Literature Cited, p. 430.

in order to cover a broad cross section of the group. Seventy-eight cultures, of which 51 were obtained from sugar beets and 27 from potatoes, were selected from several hundred isolations, attempt being made to include those from diverse geographical locations. The list of isolates with the laboratory numbers assigned is given in table 1. These organisms are referred to as isolates rather than strains, the latter term being reserved for use after distinctive characteristics have been established.

TABLE 1.—*Sources of isolates of Rhizoctonia solani used in present investigations*

Isolate no.	Host	Location	By whom isolated	Year of isolation
R-50	Sugar beet	Rocky Ford, Colo	D. Stewart	1927
R-51	do	Fort Collins, Colo	LeClerg	1924
R-52	do	Chino, Calif	D. Stewart	1927
R-53	do	East Grand Forks, Minn	LeClerg	1930
R-54	do	do	do	1930
R-55	do	do	do	1930
R-56	do	do	do	1930
R-57	do	do	do	1930
R-58	do	do	do	1930
R-59	do	do	do	1930
R-60	do	do	do	1930
R-61	do	Chaska, Minn	do	1930
R-62	do	do	do	1930
R-63	do	Worthington, Minn	do	1930
R-64	do	Oslo, Minn	do	1930
R-65	do	do	do	1930
R-66	do	do	do	1930
R-67	do	Mankato, Minn	do	1930
R-68	do	do	do	1930
R-69	do	do	do	1930
R-70	do	Oslo, Minn	do	1930
R-71	do	do	do	1930
R-72	do	Mankato, Minn	do	1930
R-73	do	do	do	1930
R-74	do	do	do	1930
R-75	do	Oslo, Minn	do	1930
R-76	do	do	do	1930
R-78	do	Mankato, Minn	do	1930
R-80	do	Oslo, Minn	do	1930
R-81	do	do	do	1930
R-82	do	do	do	1930
R-83	do	do	do	1930
R-84	do	do	do	1930
R-85	do	do	do	1930
R-88	do	Mankato, Minn	do	1930
R-93	do	Colorado	D. Stewart	1925
R-94	do	Michigan	J. E. Kotila	1930
R-95	do	do	do	1930
R-96	do	do	do	1930
R-97	do	do	do	1930
R-98	do	do	do	1930
R-99	do	do	do	1930
R-100	do	Ohio	do	1930
R-101	do	Colorado	D. Stewart	1925
R-102	do	California	do	1927
R-103	do	Michigan	J. E. Kotila	1930
R-104	do	do	do	1930
R-105	do	do	do	1930
R-106	do	do	do	1930
R-109	do	Chaska, Minn	LeClerg	1930
R-110	Potato	St. Paul, Minn	do	1930
R-114	do	Moorhead, Minn	do	1930
R-115	do	Guthrie, Minn	do	1931
R-116	do	Fosston, Minn	do	1931
R-117	do	Clearbrook, Minn	do	1931
R-118	do	Fosston, Minn	do	1931
R-119	do	Grand Forks, N.Dak	do	1931
R-121	do	Glyndon, Minn	do	1931
R-122	do	do	do	1931
R-123	do	do	do	1931
R-124	do	St. Agatha, Canada	do	1931
R-125	do	do	do	1931
R-126	do	Dilworth, Minn	do	1931
R-127	do	do	do	1931
R-128	do	do	do	1931
R-129	do	Moorhead, Minn	do	1931
R-130	do	do	do	1931

TABLE 1.—*Sources of isolates of Rhizoctonia solani used in present investigations*—Continued

Isolate no.	Host	Location	By whom isolated	Year of isolation
R-131	Potato	Moorhead, Minn	H. Darling	1931
R-132	do	Dilworth, Minn	LeClerg	1931
R-133	Sugar beet	East Grand Forks, Minn	do	1931
R-134	Potato	Grand Forks, N.Dak	do	1931
R-135	do	Dilworth, Minn	do	1931
R-136	do	do	do	1931
R-137	do	Glyndon, Minn	do	1931
R-138	do	do	do	1931
R-139	do	do	do	1931
R-140	do	Laramie, Wyo	G. H. Starr	1932
R-141	do	do	do	1932

EXPERIMENTAL RESULTS

The experimental results of this investigation are presented under two heads: (1) Physiological Studies, in which data are given on differences in cultural and other reactions of the isolates, and (2) Pathogenicity Studies, in which are summarized pathogenicity tests of isolates on seedlings of various hosts and mature roots of sugar beets.

PHYSIOLOGICAL STUDIES

RATE OF GROWTH

The rate of growth for a number of isolates was obtained by measuring the increase in diameter of colonies on potato-dextrose agar and malt-extract agar at 26° to 28° C. Two cultures, sometimes three, were measured 72 hours after inoculation. The average increase per day was determined from these data and is given in table 2.

TABLE 2.—*Average daily growth of isolates of Rhizoctonia solani on 2 different media*

Isolate no.	Average daily growth on—		Isolate no.	Average daily growth on—	
	Potato-dextrose agar	Malt-extract agar		Potato-dextrose agar	Malt-extract agar
	Mm	*Mm*		*Mm*	*Mm*
R-50	11.0	16.8	R-68	23.6	22.8
R-95	16.3	8.3	R-65	24.0	23.1
R-105	20.5	22.0	R-73	24.0	18.3
R-61	21.0	21.0	R-59	24.3	18.0
R-84	21.5	22.1	R-53	24.5	22.8
R-97	21.5	22.0	R-110[1]	24.5	17.8
R-62	21.6	19.5	R-100	24.6	19.6
R-72	21.6	15.6	R-51	24.8	22.1
R-78	22.0	18.6	R-56	25.0	23.3
R-67	22.1	20.3	R-106	25.1	26.5
R-101	22.1	21.0	R-74	26.3	21.8
R-57	22.3	20.5	R-54	28.6	18.6
R-85	22.5	23.3	R-96	29.5	27.8
R-55	22.6	26.3	R-99	29.5	26.8
R-66	22.6	21.5	R-114[1]	29.8	20.3
R-69	23.0	22.0	R-52	30.1	31.6
R-80	23.0	18.8	R-94	30.5	34.1
R-60	23.1	18.0	R-102	30.7	36.0
R-63	23.1	17.8			
R-103	23.1	23.1	Average	23.8±0.42	21.7±0.54
R-58	23.6	15.0			

[1] Obtained from potatoes; the other isolates were obtained from sugar beets.

Marked differences in the rates of growth existed among the isolates. It is to be noted that the rate of growth of an isolate is influenced by the medium upon which it is grown. For example, R–54 grew more rapidly on potato-dextrose agar than on malt-extract agar, whereas R–50 grew more rapidly on the latter. Isolate R–54 showed the greatest average difference, 10.0 mm, in daily growth rate on the two media. Taking all isolates into consideration, the average daily growth was 23.8 mm on potato-dextrose agar and 21.7 mm on malt-extract agar. While the results point definitely to physiological differences among the isolates, it is doubtful if separations could be made on this basis.

Effect of Temperature on Rate of Growth

The effect of temperature on the rate of growth of the mycelium of a number of isolates on potato-dextrose agar was determined. Petri dishes of uniform size were selected and sterilized, and 10 cc of sterile potato-dextrose agar was poured into each dish. The agar, after hardening, was inoculated with mycelial inoculum of uniform size, placed near the center of the dish. Three dishes of each isolate were placed at each of the following temperatures: 1°, 5°, 8°, 15°, 20°, 25°, 30°, 35°, and 40° C. The temperature of the chambers varied approximately 1°. The measurements from which the averages were obtained were made 60 hours after inoculation. The individual readings from a plate seldom varied more than 1 mm from the mean of three replicates.

The results of this test are summarized in table 3. There is indication that the isolates differ as to cardinal points of temperature. It is interesting to note that at 8° C. growth of R–53, R–62, R–103, and R–63 (sugar-beet isolates) was markedly less than that of R–114 (potato isolate), whereas at 35° the four sugar-beet isolates grew a trace and the potato isolate failed to grow at all.

Table 3.—*Effect of temperature on growth of 5 isolates of Rhizoctonia solani on potato-dextrose agar*

[Results given as colony diameter (in millimeters) of colonies 60 hours after inoculation]

Temperature (°C.)	Average colony diameter				
	R–53	R–62	R–103	R–114 [1]	R–63
	Mm	*Mm*	*Mm*	*Mm*	*Mm*
1	0	0	0	0	0
5	0	0	0	1.0	0
8	1.6	3.6	(2)	11.3	2.6
15	30.3	23.3	28.3	30.6	11.3
20	64.6	61.3	68.6	68.6	55.3
25	81.0	79.0	73.3	77.3	74.6
30	80.0	75.6	78.0	66.3	80.3
35	(2)	(2)	(2)	0	(2)
40	0	0	0	0	0

[1] Obtained from potatoes; the others were obtained from sugar beets. [2] Trace.

All the isolates grew well between 20° and 30° C., but none grew at 40°. The optimum temperature was between 25° and 30° for the sugar-beet isolates and between 20° and 25° for the potato isolate. Low temperature (1°) appeared to inhibit growth without causing permanent injury to the fungus. Some cultures were kept at this

temperature for 4 weeks without visible signs of growth, but when transferred to a higher temperature (25°) promptly resumed growth.

These results agree in general with previous reports on the growth-temperature relations of different cultures of this fungus. Matsumoto (*12*), working with three "strains" (P1, potato; P4, potato; H, *Habenaria* sp.), found the optimum temperature to be about 24° C. Wellman (*25*) found that cabbage *Rhizoctonia* grew best at 23° to 27°, while Walker (*23*), in investigating the "soreshin" disease of cotton, found the optimum for his strain to be between 27° and 29°, the maximum at 38°, and the minimum between 7° and 11°. Monteith and Dahl (*14*), in testing nine cultures of *Rhizoctonia solani*, found the optimum temperature for growth to be 25° to 30°. Gratz (*8*) states that the optimum temperature for *R. solani* from cabbage is 24°. Newton (*16*), working with a potato culture, found the minimum temperature to be 6°, the optimum 25°, and the maximum 32°. Müller (*15*) reported a temperature range of 4.5° to 30.8° and an optimum of 24° for the *Hypochnus solani* (*Rhizoctonia solani*) that he investigated. Richards (*19*) found that growth occurred through a range of temperatures from 4.6° to 32.5°, with an optimum between 25° and 27°.

Relation of Soil Temperature to Infection

Edson (*3*) states that "in the case of the Rhizoctonia root-rot of the sugar beet, soil temperature is a more important factor than soil texture", and that frequently "beets completely recovered during the cooler weather which set in a few days after the inoculations were made."

Experimental data from plants grown under controlled conditions were obtained to determine the effect of soil temperature on the incidence and development of root rot of sugar beets caused by *Rhizoctonia solani*. One of the isolates (R–62) obtained from sugar beets was used in these tests. Soil cans of the temperature tanks were filled with equal quantities of steamed soil, and, to insure comparable results, effort was made to maintain uniform moisture in the cans throughout the experiment. Tap water was added to the cans needing it; those at the higher temperatures required considerable each day, while those at lower temperatures required less. The figures given for soil temperatures are the average daily readings obtained from a thermometer thrust to a depth of 2 inches in the soil at the center of the can. Some deviations in temperature due to surface effects are to be expected, although the readings from which the averages were obtained seldom varied more than 1° from the mean. Small sugar-beet roots (about 1½ to 2 inches in diameter) were planted in the steamed soil, where they were allowed to grow until a number of leaves had been produced. After this the cans were allowed to stand 24 hours at the various temperatures before the roots were inoculated by placing a small quantity of grain inoculum [4] close to each root, about 1 inch below the surface of the soil.

From the experiment made in 1932 (table 4), it was found that *Rhizoctonia solani* (R–62) required an average period of 6 days at 25° C. and 10 days at 33.1° to kill sugar beets, while the period was

[4] For the sake of brevity, when cultures of the pathogene grown on agar medium in Petri dishes were used, the inoculum is referred to as "agar inoculum"; similarly, cultures grown on steamed oat and wheat kernels are referred to as "grain inoculum".

lengthened greatly at lower temperatures. It may be noted that the time required for the pathogene to kill all inoculated plants increased with decrease of soil temperature below the optimum. At 15° the rate of decay was exceedingly slow, only about 50 percent of the total tissue of each root having been destroyed at the time the experiment was terminated.[5] All uninoculated plants remained free from disease.

TABLE 4.—*Effect of soil temperature on root-rot of sugar beets, caused by Rhizoctonia solani (R–62)*

[Results are stated as averages based on 12 plants at each temperature in experiment 1 and on 6 plants in experiment 2]

Average soil temperature (°C.)	Experiment 1 (roots inoculated Jan. 18, 1932)		Experiment 2 (roots inoculated Jan. 28, 1933)	
	Period before all plants were dead	Decay produced	Period before all plants were dead	Decay produced
	Days	*Percent*	*Days*	*Percent*
15.0	>28	50		
17.8	28	100		
18.1			>15.0	40.8
18.4	26	100		
19.0	23	100		
20.7	18	100		
22.0			>15.0	79.2
24.3			>15.0	94.2
25.0	6	100		
27.0			>15.0	95.8
30.9			12.8	100.0
33.1	10	100		
34.7			15.0	100.0

A second experiment to determine the effect of soil temperature on root rot was made in 1933, but was terminated before all plants at the different temperatures had been killed. The results are summarized in table 4. The average time that elapsed between inoculation and death of 6 roots was 12.8 ± 2.4 days at 30.9° C., and 15.0 ± 4.9 days at 34.7°. Because of the high probable error at 34.7°, it is doubtful whether the difference between the number of days at this temperature and at 30.9° is significant. These data agree with the results of 1932 and show that *Rhizoctonia solani* (R–62) is most destructive at the higher soil temperatures. At temperatures below the optimum, the amount of decay at harvest decreased with decrease in temperature, and considerably more time would have been required to rot the roots completely at these temperatures. All uninoculated plants remained free from disease.

EFFECT OF HYDROGEN-ION CONCENTRATION ON GROWTH ON ARTIFICIAL MEDIA

The growth of five isolates of *Rhizoctonia solani* was studied on potato-dextrose agar adjusted to different hydrogen-ion concentrations determined by means of the quinhydrone electrode. The quantity of medium in each Petri dish was uniform (10 cc), and dishes

[5] In this experiment and in many others reisolations were made from affected plants, and the organism obtained was compared with the original one used for inoculation. Pure cultures often were obtained directly from the platings. In no case was it impossible to recover the fungus, and on comparison close greement with the original isolates was found.

of uniform size were used. The following isolates from sugar beets were used: R–53, R–61, R–63, and R–67; and a potato isolate, R–114.

The results given in table 5 show that with three exceptions the potato isolate (R–114) grew more abundantly at all hydrogen-ion concentrations than did any of the four sugar-beet isolates. All isolates grew best on an acid medium, although the optimum hydrogen-ion concentration is not the same for all. The potato isolate (R–114) and three sugar-beet isolates (R–67, R–61, and R–63) grew best at pH 6.2, as indicated by diameter of the colonies, while the other sugar-beet isolate (R–53) grew best at pH 5.6. Rabinovitz-Sereni (*18*), Gratz (*8*), Weber (*24*), and Samuel and Garrett (*21*) found the optimum hydrogen-ion concentration for their strains to be between pH 6.5 and 7.0.

TABLE 5.—*Average diameters of duplicate colonies of 5 isolates of Rhizoctonia solani, on potato-dextrose agar at different hydrogen-ion concentrations, at the end of 2 days*

Hydrogen-ion concentration (pH)	Average colony diameter				
	R–114[1]	R–67	R–61	R–63	R–53
	Mm	*Mm*	*Mm*	*Mm*	*Mm*
3.2	51.5	30.5	25.0	25.5	44.0
4.0	55.5	33.0	30.5	27.5	53.0
4.6	59.5	37.0	36.5	29.0	54.0
5.2	64.5	41.0	42.5	31.0	58.0
5.6	73.0	48.0	47.5	33.0	63.0
6.2	79.5	54.5	50.5	35.0	57.5
7.0	66.0	28.5	46.5	29.5	43.0
8.0	40.0	25.5	42.0	27.0	39.5
9.1	29.0	22.5	38.5	16.0	34.0

[1] Obtained from potatoes; the others were obtained from sugar beets.

CONCLUSIONS

The preceding experiments and many others not detailed, in which cultures were compared under controlled conditions, showed definitely that the isolates were consistently different in color, growth form, type of sclerotia produced, rate of growth, and reaction to temperature and hydrogen-ion concentration. These results are of the same type as those of other workers who have maintained that such differences indicate the existence of distinct strains within *Rhizoctonia solani*.

PATHOGENICITY STUDIES

TESTS WITH SUGAR-BEET SEEDLINGS

The isolates tested having given such strong evidence of differences under conditions of artificial culture, tests to determine degree of pathogenicity and host range were undertaken with certain isolates. The methods used by Edson (*3*) were followed in general, and in some preliminary experiments various procedures were compared. In a preliminary experiment started January 15, 1931, sugar-beet plants of three stages of development (4-leaf, 5- to 6-leaf, and 8- to 10-leaf) were available for inoculation. These plants had been grown from pasteurized seed planted in steamed soil at weekly intervals. The seedlings were transplanted to 6-inch pots of steamed

soil and inoculated in two ways: In one set, the soil was inoculated; in the other, the roots were inoculated directly by placing agar inoculum in contact with them.

Infection resulted from the use of infested soil, regardless of whether the roots were wounded or not. The smaller plants died earlier than the larger ones, the time required for death being, in general, directly proportional to the size of the root. Similar results were obtained by direct inoculation of roots growing in steamed soil, in which case wounded plants died in one-third to one-half the time required for the unwounded ones. The results of both types of inoculation show that, in general, plants are susceptible in all stages of development when inoculated by either method, but those inoculated directly die much sooner than those growing in inoculated soil. The fact that unwounded plants are killed agrees with the observation of Edson (*3*), who states that "The fungus is capable of attacking its host at any time after germination", and "young beets 4 or 5 weeks old were readily killed * * * when no wound was made." These tests indicate that infection results readily either from direct inoculation or as a result of adding the pathogene to the soil.

Tests with Large Sugar-Beet Plants

In field experiments made at University Farm, St. Paul, Minn., in August 1931, several different methods of inoculation were tested on large sugar-beet plants. These included the use of agar inoculum and grain inoculum on wounded and unwounded plants at the base of the petiole, at the ground line, and 1 inch below the surface of the soil. Wounds were made with a knife in imitation of cultivator injury. All inoculations were made in the late afternoon, and plants with petiole inoculations were watered thoroughly and covered with wet newspapers until the following morning, to provide increased humidity for approximately 12 hours.

The results of these inoculations are summarized in table 6. They show definitely that inoculations at the base of the petiole with agar or grain inoculum did not give satisfactory results, although grain inoculum produced a higher percentage of infection than agar inoculum. These differences probably are to be attributed to the more rapid drying out of the agar inoculum. Grain inoculum applied either 1 inch below the soil or at the ground line produced more infection than at the base of the petioles; greatest infection occurred in roots inoculated at the ground line. With all inoculation methods, infection usually was heavier in wounded roots. Since inoculation of wounded roots at the ground line with grain inoculum resulted in 100 percent infection in two experiments, this procedure was adopted for all future experiments.

TABLE 6.—*Incidence and development of root rot in sugar beets, inoculated in the field by various methods with Rhizoctonia solani (R–62), at University Farm, St. Paul, Minn.*

Inoculum	Place of inoculation	Treatment of plants	Plants inoculated			Plants infected			Average of plants infected [1]
			Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3	
			Number	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Agar	Base of petioles	Wounded	17		27	0		0	0
Do	do	Not wounded	14		22	7.1		0	3.5
Grain	do	Wounded	17	10	26	47.1	20.0	76.9	48.0
Do	do	Not wounded	17	10	26	70.6	60.0	46.2	58.9
Do	1 inch below soil	Wounded	13	10	25	100.0	90.0	92.3	94.1
Do	do	Not wounded	15	10	26	100.0	50.0	92.3	80.8
Agar	do	Wounded	16		28	100.0		64.3	82.1
Do	do	Not wounded	19		27	94.7		25.9	60.3
Do	Ground line	Wounded	16		27	56.3		18.5	37.4
Do	do	Not wounded	17		26	47.1		23.1	35.1
Grain	do	Wounded	18		18	100.0		100.0	100.0
Do	do	Not wounded	20		17	85.0		100.0	92.5

[1] None of the control plants became infected

COMPARATIVE PATHOGENICITY OF ISOLATES

Differences in pathogenicity of cultures of *Rhizoctonia solani* have been noted by a number of investigators (*2*, *4*, *8*, *12*, *17*, *20*, *22*, *26*, and others), and several have tested the pathogenicity to other hosts of cultures isolated from sugar beets. Edson and Shapovalov (*4*) reported a number of such cultures of *R. solani* as being capable of causing stem lesions on potatoes; two cultures from beets were found to be parasitic on pine seedlings by Gravatt (*9*) and Hartley (*10*); and a sugar-beet culture was found by Peltier (*17*) to be pathogenic to carnation seedlings, cuttings, and old plants. Wiant (*26*) tested the pathogenicity of two cultures from beets and found them capable of causing damping off of coniferous seedlings and peas, cabbage, eggplant, pepper, and tomato. As far as the writer knows, no one has tested the pathogenicity of potato isolates on mature sugar-beet roots; therefore pathogenicity tests were made in the laboratory, greenhouse, and field.

LABORATORY TESTS WITH SUGAR-BEET SLICES

Pathogenicity tests on slices of sugar-beet roots were made in the aboratory during January 1931. Roots were washed, surface-sterilized with 1 : 1,000 solution of mercuric chloride, and rinsed in sterile distilled water, after which slices approximately 5 mm in thickness and 40 mm in diameter were cut from them. Two slices, in some cases three, of uniform size, after inoculation with agar inoculum, were placed in Petri dishes, and the amount of rot was noted 10 days later. The amount could not be determined from the inoculated surface alone, since the entire surface often was discolored, although the rot extended only a few millimeters into the tissue. Therefore each slice was cut in two at the point of inoculation, and each piece was compared with a set of standards which had been set up previously. Personal prejudice in making determinations was avoided by making all readings as blind notations, without knowledge of the isolates concerned.

It is recognized that results from an experiment of this kind, made with a limited number of beet slices, must take into account the variation which may exist among slices, but the data in table 7 and in figures 1 and 2 show that there are wide differences in the amount of rot produced by the sugar-beet isolates (for example, isolate R–109 caused no rot, whereas R–80 caused 93.3 percent), and that the isolates from potato (R–110 and R–114) produced only a trace of rot.

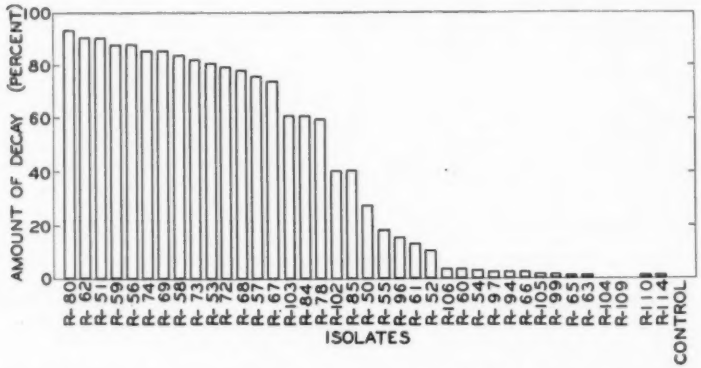

FIGURE 1.—Percentage of rot caused by some isolates of *Rhizoctonia solani* inoculated in slices of sugar beets under controlled conditions. All isolates used, except R–110 and R–114 (from potato), were obtained from sugar beet.

TABLE 7.—*Results of inoculating sugar-beet slices with isolates of Rhizoctonia solani from sugar beets and potatoes*

Source of isolates	Isolates tested	Average decay
	Number	*Percent*
Sugar beet	36	[1] 37.8
Potato	2	[2]
Control		0

[1] The percentage of decay ranged from 0 to 93.3 percent.
[2] Trace.

INOCULATIONS OF SUGAR-BEET ROOTS

Extensive field experiments were undertaken to test more thoroughly the pathogenicity of the two groups of isolates. A large number of sugar-beet roots were inoculated with 2 potato and 9 sugar-beet isolates in the field at University Farm, St. Paul, Minn., in 1931. Results are given in table 8. No decay resulted in plants inoculated with isolates from potatoes (R–110 and R–114), whereas all isolates from sugar beets caused decay. The amount of rot produced by the sugar-beet isolates differed considerably, some causing twice as much as others. This is illustrated by R–52 and R–69, the former causing 15.0 percent rot and the latter 31.6 percent.

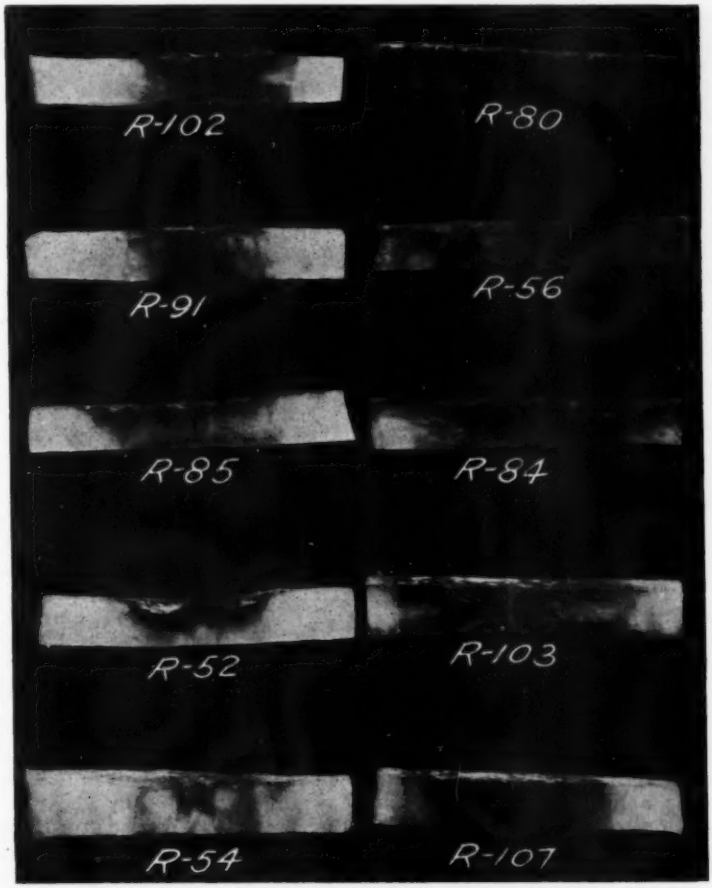

FIGURE 2.—Typical examples from experiment in which beet slices were inoculated (fig. 1), showing differences in amount of decay produced by some of the isolates.

TABLE 8.—*Results of inoculating sugar beets in the field with isolates of Rhizoctonia solani at University Farm, St. Paul, Minn., 1931*

Isolate no.	Roots inoculated	Roots diseased		Average decay in affected roots	Isolate no.	Roots inoculated	Roots diseased		Average decay in affected roots
	Number	*Number*	*Percent*	*Percent*		*Number*	*Number*	*Percent*	*Percent*
R-114 [1]	31	0	0	0	R-53	27	26	96.3	24.0
R-110 [1]	26	0	0	0	R-62	27	27	100.0	25.7
R-106	28	1	3.6	15.0	R-51	25	25	100.0	28.0
R-52	25	8	32.0	15.0	R-65	26	26	92.8	29.0
R-103	32	27	84.4	19.8	R-69	31	28	90.3	31.6
R-50	28	15	53.6	20.7	Control [2] 432	0	0	0	0

[1] Obtained from potatoes; the others were obtained from sugar beets.
[2] Control plants were treated in the same manner as inoculated plants, except that the grain substratum did not contain any organism.

To test the reaction of sugar beets to a larger number of potato isolates, with a few isolates from sugar beets for comparison, the following inoculations were made. Roots about 1½ to 2 inches in diameter were transplanted to steamed soil in 6-inch pots and grown until an abundance of leaf tissue had been produced. These rapidly growing roots were inoculated January 16, 1932. Five wounded roots were uniformly inoculated with grain inoculum of each isolate, and five uninoculated roots were maintained as controls.

The results of this test are given in table 9. None of the 25 isolates from potato caused infection, whereas all the sugar-beet isolates caused root rot, 93.3 percent of the roots inoculated becoming infected. The average percentage of rot produced was 63.7 percent, the individual readings ranging from 5.3 to 88.0 percent.

TABLE 9.—*Results of inoculating sugar-beet roots with various isolates of Rhizoctonia solani in the greenhouse*

Source of isolates	Isolates tested	Plants inoculated	Roots infected	Average decay in crown
	Number	*Number*	*Percent*	*Percent*
Sugar beet	6	30	93.3	63.7
Potato	25	125	0	0
Control		[1] 5	0	0

[1] Control plants were treated in the same manner as the inoculated plants, except that the grain substratum did not contain any organism.

More extensive inoculations were made during the summer of 1932, to compare the pathogenicity of a large number of isolates from both sugar beet and potato. These experiments were made at Grand Forks, N.Dak., St. Paul, Minn., and Waseca, Minn., which are widely separated localities with considerable differences in environmental conditions.

The data obtained are given in table 10 and shown graphically in figure 3. A view of a portion of the test plot at University Farm is shown in figure 4, *A*. The striking contrast between the results from potato and sugar-beet isolates is shown in figure 4, *B*. The tests point out clearly that the isolates from sugar beet caused rot at all the stations, while those from potato were nonpathogenic to sugar beets. The potato isolates were collected over a wide geographical area in Minnesota and Manitoba, except R–140 and R–141, which were collected in Wyoming. That these two isolates were also nonpathogenic is of considerable interest. These results suggest strongly that potato isolates from other geographical localities may be nonpathogenic for sugar beets. This point is being investigated further. The experiment also gives some indication of the loss *Rhizoctonia* may cause to sugar beets. The average weight per individual root was reduced as a result of the action of the pathogene (fig. 3). The decrease below the control was approximately in direct proportion to the amount of rot produced.

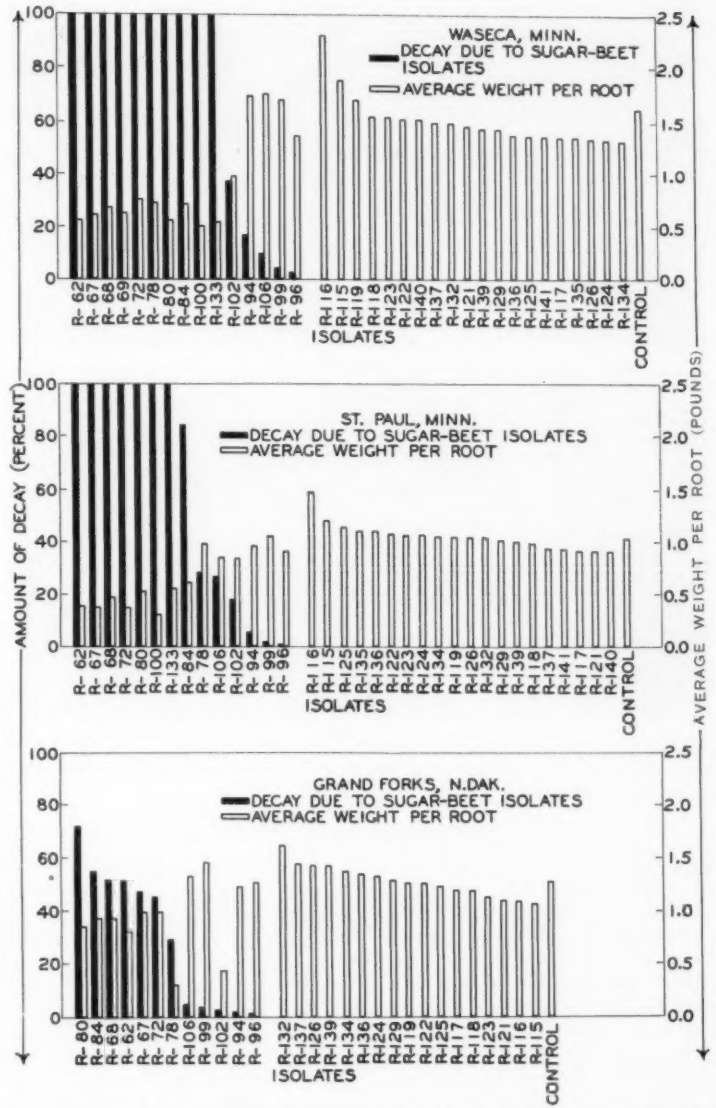

FIGURE 3.—Amount of decay of sugar-beet roots caused by isolates of *Rhizoctonia solani* from potato and sugar beet, and average weight per root. Plots located at Waseca, Minn., St. Paul, Minn., and Grand Forks, N.Dak., in 1932. It is to be noted that no rot was caused by any of the potato isolates (R–115 to R–141) at any of the stations.

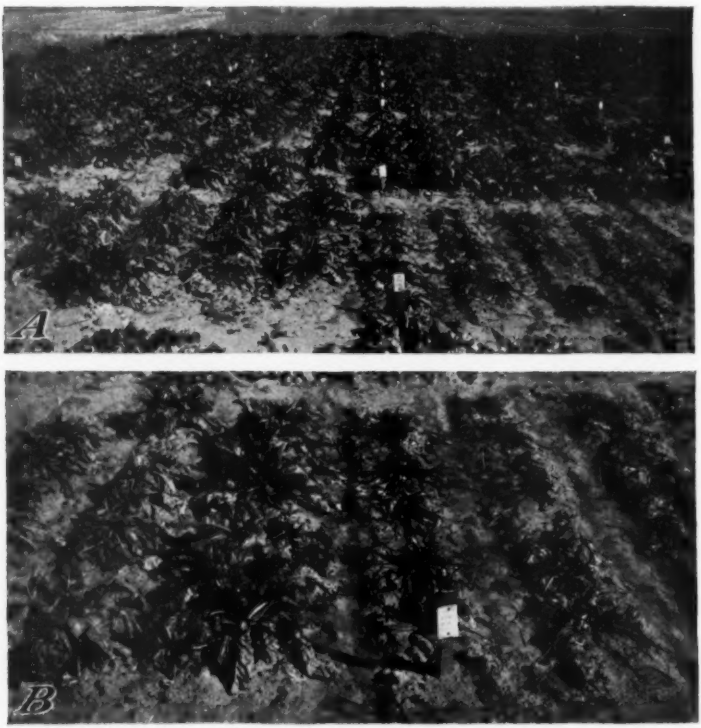

FIGURE 4.—Root-rot inoculation plots at University Farm, St. Paul, Minn., in 1932. *A*, Plants in right center foreground and in right center background were inoculated with isolates from sugar beets (all plants in plots are dead). Others either were inoculated with potato isolates or were controls. *B*, Close-up of 2 plots, 1 (right) in which plants were inoculated with a sugar-beet isolate (R–72), and 1 (left) in which a potato isolate (R–121) was used.

TABLE 10.—*Results of inoculating sugar beets in the field with isolates of Rhizoctonia solani at Grand Forks, N.Dak., St. Paul, Minn., and Waseca, Minn., in 1932*

Station	Source of isolate	Isolates tested	Roots inoculated	Total weight of roots	Average weight per root	Average decay
		Number	*Number*	*Pounds*	*Pounds*	*Percent*
Grand Forks	Sugar beet	12	479	451.4	0.94	33.6
	Potato	17	757	952.2	1.26	0
	Control		[1] 351	443.5	1.26	0
St. Paul	Sugar beet	14	480	362.5	.76	58.9
	Potato	20	630	662.8	1.05	0
	Control		[1] 357	367.4	1.03	0
Waseca	Sugar beet	15	354	364.3	1.03	50.8
	Potato	20	554	819.5	1.48	0
	Control		[1] 190	296.6	1.56	0
Average	Sugar beet				.91	47.8
	Potato				1.26	0
	Control				1.28	0

[1] Control plants were treated in the same manner as the inoculated plants, except that the grain substratum did not contain any organism.

RELATION OF ISOLATES TO DAMPING OFF OF SEEDLINGS

Having demonstrated that isolates of *Rhizoctonia solani* from potato are nonpathogenic to large sugar-beet roots, the relation of these isolates to the damping off of sugar beets and some other host plants was studied.

Sugar beets.—In a preliminary experiment made in 1931, sugar-beet seed balls[6] were planted in 4-inch pots of steamed and infested soil. Thirty seed balls were planted in each pot. The soil was infested with an isolate by mixing it with grain inoculum of the pathogene. For the controls, sterilized grain only was mixed with soil. Seedling counts were made 14 days after planting. The average seedling stand in the control pots was taken as 100 percent, and stands in infested pots were put on a percentage basis from this. The number of seedlings emerged was taken as an index of the virulence of an isolate.

It is evident from the results given in table 11 that all the isolates of *Rhizoctonia solani* did not attack sugar-beet seedlings with the same degree of virulence. Isolates R–110 and R–114 (potato) are outstanding in that they were only very weakly virulent, whereas R–51, R–63, R–65, R–99, and R–106 (sugar beet) were strongly virulent, each causing 100 percent damping off.

TABLE 11.—*Results of preliminary inoculations of sugar beet seedlings with various isolates of Rhizoctonia solani, showing the percentage of damping off as compared to the control*

Isolate no.	Total emergence in 3 pots	Average damping off	Isolate no.	Total emergence in 3 pots	Average damping off
	Number	*Percent*		*Number*	*Percent*
R–114[1]	122	0.0	R–51	0	100.0
R–110[1]	102	9.7	R–63	0	100.0
R–50	29	74.3	R–65	0	100.0
R–53	21	81.4	R–99	0	100.0
R–103	12	89.4	R–106	0	100.0
R–69	7	93.8	Control	113	.0
R–52	4	97.5			

[1] Obtained from potatoes; the others were obtained from sugar beets.

In a more extensive experiment, made also in 1931, the susceptibility of sugar-beet, table-beet, and alfalfa (*Medicago sativa* L.) seedlings to a number of isolates from potato and sugar beet was determined in the greenhouse. The soil was infested as described above for the preliminary sugar-beet experiment, and seedling counts were made about 14 days after planting. The rate of seeding per pot varied according to the size of the seed: 30 seed balls of sugar beets, 35 of table beets, and 200 of alfalfa.

The results of the sugar-beet experiment (table 12) are shown graphically in figure 5. All sugar-beet isolates were strongly viru-

[6] Use of sugar-beet seed balls introduces certain difficulties in securing concordant results, because the seed balls contain a variable number of embryos, which results in irregular emergence of sprouts, and the seedling stand may be seriously affected because of the seed transmission of *Phoma betae* (Oud.) Frank. The following methods were adopted by the writer in order to standardize the tests as much as possible: Seed balls were screened and only those of approximately uniform size were used. Stand counts were made when emergence was approximately complete. Sugar-beet seed balls, as well as those of table beet, mangel-wurzel, and Swiss chard, were treated by the pasteurization method of Edson (*3*), to eliminate as far as possible infection from the seed-borne pathogene *P. betae*.

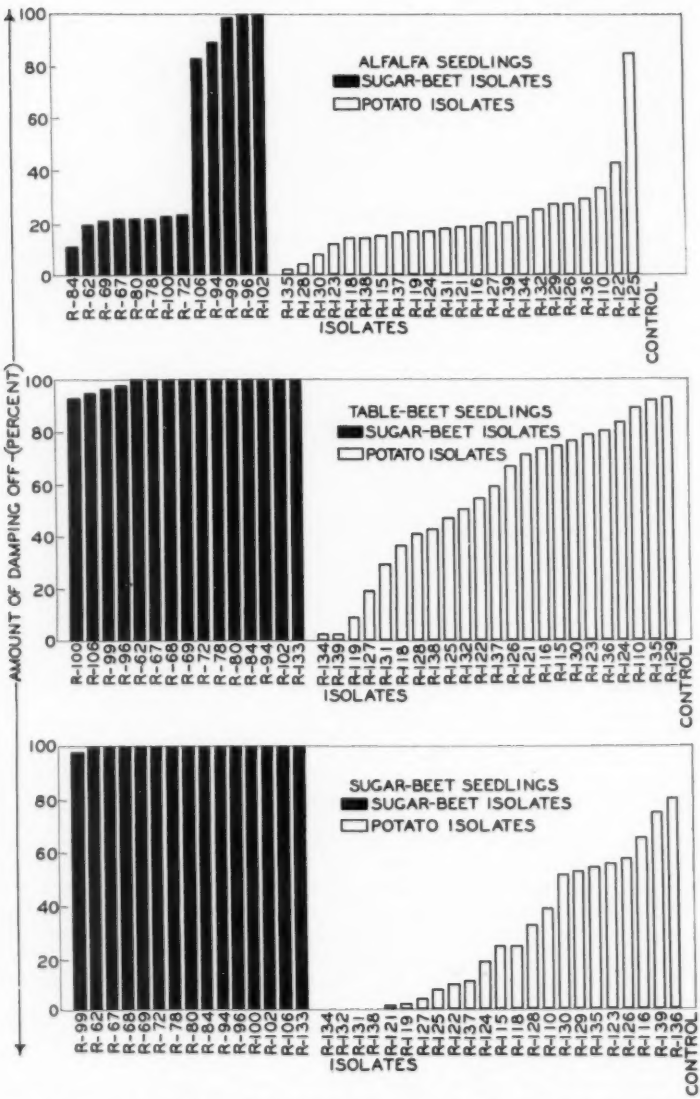
AMOUNT OF DAMPING OFF (PERCENT)
ALFALFA SEEDLINGS
SUGAR-BEET ISOLATES
POTATO ISOLATES
R-84 R-62 R-69 R-67 R-80 R-78 R-100 R-72 R-106 R-94 R-99 R-96 R-102 R-135 R-128 R-130 R-123 R-118 R-138 R-115 R-137 R-127 R-119 R-124 R-131 R-121 R-116 R-132 R-139 R-126 R-136 R-110 R-122 R-125 CONTROL
ISOLATES
TABLE-BEET SEEDLINGS
SUGAR-BEET ISOLATES
POTATO ISOLATES
R-100 R-106 R-99 R-96 R-62 R-67 R-68 R-69 R-72 R-78 R-80 R-84 R-94 R-102 R-133 R-134 R-139 R-119 R-127 R-131 R-118 R-128 R-138 R-125 R-132 R-122 R-137 R-126 R-121 R-116 R-115 R-130 R-123 R-136 R-124 R-110 R-135 R-129 CONTROL
ISOLATES
SUGAR-BEET SEEDLINGS
SUGAR-BEET ISOLATES
POTATO ISOLATES
R-99 R-62 R-67 R-68 R-69 R-72 R-78 R-80 R-84 R-94 R-96 R-100 R-102 R-106 R-133 R-134 R-132 R-131 R-138 R-121 R-119 R-127 R-125 R-122 R-137 R-115 R-118 R-128 R-110 R-130 R-129 R-135 R-123 R-126 R-116 R-139 R-136 CONTROL
ISOLATES

FIGURE 5.—Amount of damping off of alfalfa, table beets, and sugar beets produced by isolates of *Rhizoctonia solani* from potato and sugar beet.

lent causing not less than 97.4 percent damping off, while the majority caused 100 percent. It is important to note that none of the potato isolates caused so much damping off; the greatest amount was caused by R–136 (80 percent), while R–139, ranking second, caused 75.5 percent decrease in stand. It appears that the isolates from potato were less virulent than those from sugar beet.

TABLE 12.—*Results of inoculating sugar beets, table beets, and alfalfa with various isolates of Rhizoctonia solani, showing the average percentage of seedlings damped off*

[Results given as averages of 5 replicates]

Host	Source of isolate	Isolates tested	Damping off	
			Range	Average
		Number	*Percent*	*Percent*
Sugar beet	Sugar beet	15	97.4–100.0	99.8
	Potato	23	0 – 80.0	28.5
	Control			0
Table beet	Sugar beet	15	93.0–100.0	92.1
	Potato	23	2.5– 92.4	50.1
	Control			0
Alfalfa	Sugar beet	13	15.6– 98.9	48.6
	Potato	23	1.6– 81.4	21.2
	Control			0

Table beets.—The capacity of isolates of *Rhizoctonia solani* to cause damping off of table-beet seedlings was also investigated. Pasteurized seed balls were planted in 4-inch pots as described for the sugar-beet-seedling test. A summary of the results obtained is given in table 12 and in figure 5. It is seen from the figure that all the isolates obtained from sugar beets were strongly virulent to table beets. Although a few (R–110, R–129, R–135) of the isolates from potato were highly virulent to table beets, the majority were less virulent. As will be seen by comparison with the results obtained with sugar-beet seedlings, some isolates from potato produced considerable damping off of sugar beets and a small amount on table beets; other isolates acted in the opposite way. Thus isolate R–139, which caused 75.5 percent of damping off of sugar beets, caused only 2.5 percent of damping off of table beets, whereas isolate R–122 caused on sugar beets and table beets, respectively, 9.7 percent and 54.5 percent of damping off.

Alfalfa.—The pathogenicity and virulence of the isolates to alfalfa seedlings is also significant. From the data presented in table 12 and figure 5 it is apparent that the various isolates of *Rhizoctonia solani* do not attack alfalfa seedlings uniformly. It appears that the isolates from sugar beet are generally less virulent to alfalfa than to sugar-beet or to table-beet seedlings, since the amount of damping off ranged from 15.6 percent with R–84 to 98.9 percent with R–96 and R–102. Greater reduction in stand was caused by many of the isolates from sugar beet than by most of those from potato. The degree of virulence of the isolates obtained from potato, like that of the sugar-beet isolates, was not uniform. Isolate R–125 was the most destructive, causing 81.4 percent of damping off, while R–135 was least destructive and caused only 1.6 percent decrease in stand.

Other hosts.—In these studies 3 isolates of *Rhizoctonia solani* were used: 1 strongly virulent (R–67), 1 intermediate in virulence (R–128), and 1 almost nonpathogenic (R–134), as indicated by their relative ability to cause damping off of sugar-beet seedlings (fig. 5). Additional tests in the greenhouse, on 11 different host plants, were made with these isolates. In these experiments 4-inch pots were filled with steamed soil infested with an isolate. Each test with an isolate and host was replicated five times. Five pots containing inoculum without a pathogene were maintained as controls for each host. The number of seeds per pot for each host was as follows: Sweetclover (*Melilotus* Mill.), 200; alfalfa, 200; turnip (*Brassica rapa* L.), 150; rutabaga (*B. campestris* L.), 150; cabbage (*B. oleracea capitata* L.), 125; tomato (*Lycopersicon esculentum* Mill.), 100; Swiss chard, 75; mangel-wurzel, 75; table beet, 70; sugar beet, 60; and pea (*Pisum sativum* L.), 25. The pots were arranged on the greenhouse bench to make five randomized blocks.

Table 13 shows the average number of seedlings per pot remaining after 13 days in the control and in the infested pots and the average percentage of damping off for each host. Since the comparative reactions of the three isolates on each host are the most important considerations, an analysis of variance (*6*, *7*) was made of the data for each host separately. Thus it could be determined whether the number of seedlings affected, for each isolate on each host, reached the significant figure. The analysis of the results with sugar-beet seedlings is given in tables 14 and 15 to illustrate the method used. The results with other host plants gave similar decisive results, the z value in every case greatly exceeding that of z at the 1-percent point.

TABLE 13.—*Comparative virulence of 3 isolates of Rhizoctonia solani on 11 different host plants*

[Results given as average number of seedlings per pot (5 replicates) and as percentage of damping off in inoculated series after 13 days]

Host inoculated	Seed planted per pot	Data for seedlings inoculated with—						Control [1] (average uninoculated seedlings per pot)	Observed value of z [2]	Difference in seedling number required for significance
		R–67		R–128		R–134				
		Average per pot	Damping off	Average per pot	Damping off	Average per pot	Damping off			
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*		*Number*
Alfalfa	200	88.9	33.3	96.8	27.4	111.8	16.2	133.4	1.4357	14.5
Cabbage	125	26.4	64.9	34.0	54.8	71.6	4.8	75.2	1.4505	18.4
Mangel-wurzel	[3] 75	12.0	88.4	83.4	19.2	95.2	7.6	103.2	2.4140	13.2
Pea	25	1.0	95.7	20.0	13.1	22.2	3.4	23.0	2.4665	2.8
Rutabaga	150	8.2	93.2	70.4	41.5	103.6	14.0	120.4	2.2839	15.6
Sugar beet	[3] 60	7.4	94.6	110.8	19.6	127.2	7.7	137.8	2.8924	10.1
Sweetclover	200	8.2	94.1	37.2	73.1	99.4	27.9	137.8	2.3029	18.3
Swiss chard	[3] 75	6.2	94.7	83.4	27.0	109.8	5.2	115.8	2.3911	14.3
Table beet	[3] 70	7.0	93.4	72.0	31.6	102.6	2.5	105.2	2.5666	10.9
Tomato	100	10.8	85.5	23.6	68.3	57.4	22.9	74.4	2.3801	8.5
Turnip	150	14.2	86.1	65.6	35.6	84.2	17.5	102.0	2.0503	15.2

[1] Considered as 100-percent stand.
[2] Calculated value for z at the 5-percent point is 0.6250 and at the 1-percent point it is 0.8919.
[3] Seed balls were used; these usually contain more than 1 seed.

TABLE 14.—*Comparison of seedling counts, after 13 days from inoculations of sugar beets with 3 isolates of Rhizoctonia solani, as compared with uninoculated control*

Inoculum	Average seedling count per pot	Comparison of isolates and control		
		R-67	R-128	R-134
R-67	7.4		+103.4	+119.8
R-128	110.8	−103.4		+16.4
R-134	127.2	−119.8	−16.4	
Control	137.8	−130.4	−27.0	−10.6

TABLE 15.—*Analysis of variance applicable to data of table 14*

Variation due to—	Sum of squares	Degrees of freedom	Variance	*z*
Blocks	520.2	4	130.05	
Treatments [1]	53,947.6	3	17,982.53	[2] 2.8924
Isolates	12,187.6	2	6,093.80	[2] 3.5026
Isolates v. no isolates	41,760.0	1	41,760.00	[2] 4.4650
Error	663.4	12	55.28	
Total	55,131.2	19		

[1] The control was considered as a treatment in comparison with the 3 isolates.
[2] Exceeds the value for *z* at the 1-percent point.

The significance to be attributed to the effect of the various treatments with organisms (control pots considered as a treatment) was determined by Fisher's *z* test. The values of *z* were obtained from the tables prepared by Fisher (*6*). By using the number of degrees of freedom ascribed to error and referring to the table of *t* prepared by Fisher (*6*), one can ascertain the number by which the standard error of the difference should be multiplied to obtain a value (number of seedlings) required for significance at the 5-percent point. Thus, in the analysis of variance of these data, 12 degrees of freedom are ascribable to error and by referring to the table of *t* one finds that the standard error is to be multiplied by 2.179; therefore in comparing differences in numbers of seedlings for each host for the isolates used and the control, differences as great or greater than 2.2 times the standard error of the difference between two means were considered significant.

In these experiments it is first necessary to know if the estimate of variance due to isolates is significantly greater than that due to error. The observed values of *z* for each of the 11 hosts exceeded the *z* value at both the 5-percent and 1-percent points, hence significant results were obtained from each host. From the values obtained for error for each host the standard error of the difference between two means was calculated.[7] The test with sugar beets is presented in some detail to illustrate method used in analysis of the results (table 14). From the analysis of variance, following the procedure outlined by Fisher (*6*), the value of *z* was found. Since this greatly exceeds the 1-percent point (indicating that differences as great as or greater than these due to chance would be found less than once in 100 times), the experiment as a whole was significant. The standard error of the

[7] Standard error difference $=\sqrt{\text{Standard error mean}\times 2}$.

difference between two means in the case of sugar beets was 4.59, and the number of seedlings required for a difference between two means to be considered significant was 10.1 seedlings. Using this value, it is to be noted that all the isolates reduced the number of beet seedlings below the number in the control significantly. Similarly the isolates differed among themselves significantly in the number of seedlings remaining in the pots after 13 days. The data show clearly that R–67 was most virulent, standing out distinctly because of the great reduction in stand produced; R–128 and R–134 were much less virulent, the latter only slightly exceeding the number taken as the level of significance. It is to be noted that these two isolates were obtained from potato.

Similar analyses were then made of the results with these isolates with the other hosts. The values obtained for z and the number of seedlings necessary in order for a difference to reach significance for these hosts are given in table 13.

Comparison of the number of seedlings emerging (table 13) for each isolate and control brings out the fact that the order of virulence of the three isolates (R–67, R–128, and R–134) was the same on rutabaga, sweetclover, Swiss chard, table beet, tomato, turnip, mangel-wurzel, and pea as it was with respect to seedlings of sugar beets, that is, R–67 was most virulent, R–128 intermediate, and R–134 the least virulent. For the remainder of the hosts tested this relationship does not exist. On alfalfa and cabbage, the reduction in number of seedlings by R–67 and R–128 did not differ significantly, but both were more virulent than R–134. On mangel-wurzel and pea, R–128 was not significantly differentiated from R–134. The data also show that R–134 significantly reduced the number of seedlings of alfalfa, rutabaga, sugar beet, sweetclover, tomato, and turnip, but did not significantly affect the stand of cabbage, mangel-wurzel, pea, Swiss chard, or table beet.

Stunting was observed in many seedlings growing infested soil (fig. 6). In order to determine the extent of this stunting, measurements were made of the height of 10 seedlings in each of 5 pots for each isolate and control. These measurements are summarized in table 16.

These data were analyzed by using the analysis-of-variance method. In this experiment it is necessary to know if the estimate of the variance attributable to isolates is significantly greater than that due to error. The value for z found (2.3916) exceeded the z value at the 1-percent level of significance; therefore it is apparent that the effect of the isolates on height of plants was in general highly significant. In this analysis of variance (table 17) the interaction of hosts by isolates was also determined, and it was found that the value of z (1.1573) also exceeded the value of z at the 1-percent point. This showed that there was a very significant differential effect of individual isolates on the hosts. The most obvious differential response is that of R–67, which caused only slight differences in height of certain hosts but completely killed all pea seedlings so that the mean height was zero. The data also show clearly that each isolate had a specific reaction on many of the hosts and that this reaction was not uniform for all.

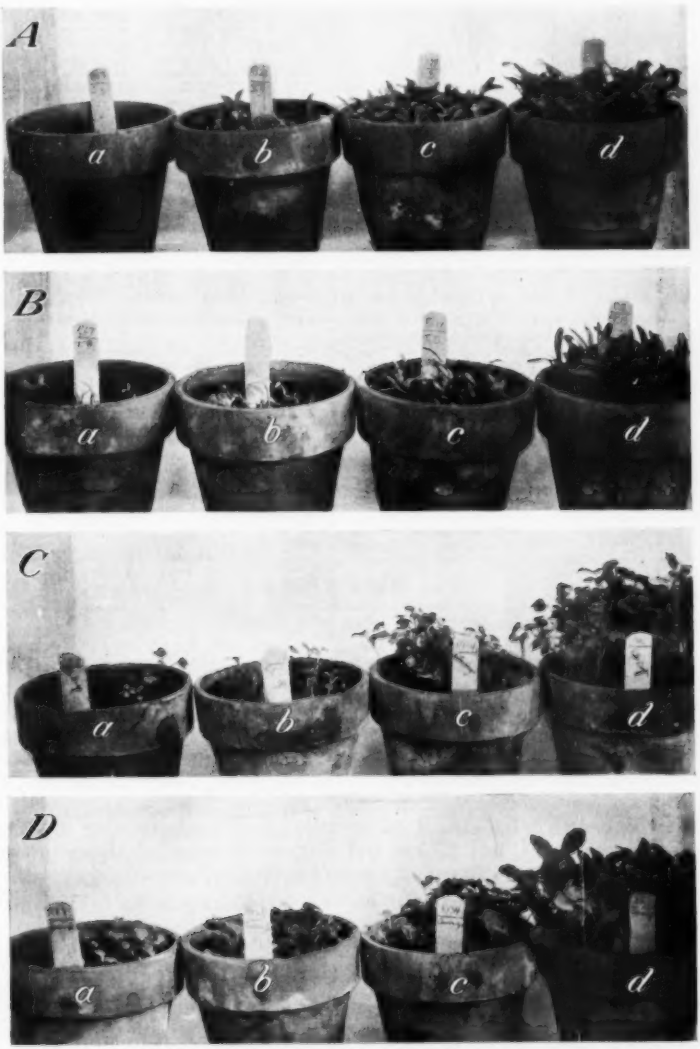

FIGURE 6.—Effect of three isolates of *Rhizoctonia solani* (*a*, R–67, obtained from sugar beet; *b*, R–128, and *c*, R–134, both obtained from potato; and *d*, control) on the height of sugar beet (*A*), table beet (*B*), rutabaga (*C*), and turnip (*D*).

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TABLE 16.—*Average height and stunting of various seedling plants when grown in soil with 3 isolates of Rhizoctonia solani (R–67, R–128, and R–134)*

Host	Data for seedling plants grown in soil infested with—						Control (average height [2] of seedling plants grown in uninfested soil)
	R–67		R–128		R–134		
	Average height [1]	Stunting	Average height [1]	Stunting	Average height [1]	Stunting	
	Mm	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
Alfalfa	50.7	3.1	51.2	2.1	50.6	3.3	52.3
Cabbage	16.0	59.7	15.0	62.2	22.3	43.8	39.7
Mangel-wurzel	16.4	43.5	20.8	28.3	28.6	1.4	29.0
Pea	0	100.0	24.6	49.6	33.0	32.4	48.8
Sugar beet	7.7	66.8	11.3	51.3	15.2	34.5	23.2
Sweetclover	9.0	69.5	8.9	69.8	19.9	32.6	29.5
Swiss chard	7.9	67.0	11.2	53.1	15.9	33.5	23.9
Table beet	12.9	32.1	10.9	42.6	14.7	22.6	19.0
Tomato	8.7	40.4	8.6	41.1	8.6	41.1	14.6

[1] Mean difference of 5.4 mm required for significance.
[2] Height of seedlings in control considered as 100 percent in calculation of stunting caused by the isolates.

TABLE 17.—*Analysis of variance applicable to data of table 16*

Variation due to—	Sum of squares	Degrees of freedom	Variance	*z*
Replicates	41.40	4	10.35	
Hosts	24,229.65	8	3,028.71	
Isolates	6,806.53	3	2,268.85	[1] 2.3916
Interaction (hosts × isolates)	4,610.77	24	192.11	[1] 1.1573
Error	2,657.30	140	18.98	
Total	38,345.65	179		

[1] Exceeds the 1 percent value for *z*.

The standard error of the difference between two means is 2.75 mm. Taking twice the standard error, for 140 degrees of freedom due to error, as the 5-percent level of significance (2.75 mm × 2.0 = 5.5 mm), mean differences in height that were as great as or greater than 5.5 mm were considered significant. These data indicate that the isolates caused stunting,[8] when compared to the height of the control plants, of seedlings of sweetclover, tomato, cabbage, sugar beet, pea, table beet, and Swiss chard. Isolates R–67 and R–128 caused stunting of mangelwurzel seedlings, but R–134 did not, while none of them caused stunting of alfalfa seedlings. In most cases R–67 caused more reduction in height than either of the other isolates.

DISCUSSION AND CONCLUSIONS

The most outstanding result of these investigations is that whereas the isolates of *Rhizoctonia solani* obtained from sugar beets were found to be capable of rotting sugar-beet roots in the field and greenhouse, the isolates obtained from potato did not cause rotting. This was true of all potato isolates tested. The two types of isolates apparently have a difference in host range. This finding is of particular significance because of the cropping systems in use in the Red River Valley

[8] The seedlings of turnip and rutabaga in inoculated pots were stunted as compared to those in the controls. This was thought to be due to the effect of crusting of the fungus and soil mixture and not to the pathogenes, since the seedlings were badly twisted beneath the soil before they emerged.

of Minnesota and North Dakota in which sugar beets often follow potatoes. From the data now available, it seems that sugar-beet rot occurring in the field, caused by *R. solani*, is not occasioned by the rhizoctonia disease which may have been present on potatoes in a previous season. It is recognized that some potato-inhabiting forms may exist that are also pathogenic to sugar-beet roots, but in the rather large number of forms from potato tested none was found pathogenic to older beet roots. Detailed study is now under way of additional material, consisting of isolations of *Rhizoctonia* from potatoes from other portions of North America and from Europe.

The physiological studies showed the same sort of differences among the isolates as have been recorded previously for *Rhizoctonia solani* and as have been taken to indicate the presence of definite strains within the species. Most previous work had not revealed definite specificity with respect to pathogenicity.

The inoculations herein reported, for the collection of isolates from sugar beets and potatoes, have shown a high degree of specificity. It is believed that the tests were extensive enough to point to the existence of a marked distinction between the two groups of isolates. The results with nine host plants used in tests of pathogenicity and virulence of the isolates to seedlings, show also that within the *Rhizoctonia solani* group further subdivisions on the basis of pathogenicity may be made. These results are interpreted to mean that there are distinct parasitic strains of *R. solani* comparable to the physiologic forms of the rusts, mildews, and other fungi. Further studies are in progress to determine more definitely the extent of physiologic specialization.

SUMMARY

There is a marked difference in rate of growth of isolates of *Rhizoctonia solani* on the same and different media.

The optimum temperature for growth on artificial media for some isolates is 25° C., while for others it is 30°. At 40° no growth occurred. Low temperature (1°) inhibits growth of some isolates without causing permanent injury.

Soil temperature of approximately 25° to 33° C. is most favorable for development of rhizoctonia root rot of sugar beets.

Five isolates tested grew over a wide range of hydrogen-ion concentrations, the optimum for one (R–53) being about pH 5.6 and pH 6.2 for the four others.

Sugar beets were found to be susceptible to *Rhizoctonia solani* at all stages of development.

There were wide differences in the ability of different isolates to rot sugar-beet slices.

Greenhouse and field inoculations prove conclusively that the sugar-beet isolates tested are pathogenic to large sugar-beet roots, while potato isolates are not.

It is concluded that rhizoctonia root rot of sugar beets is probably caused by strains of *Rhizoctonia solani* distinct pathogenically from those on potato.

Sugar-beet isolates caused a high percentage of damping off of sugar-beet and table-beet seedlings, and potato isolates considerably less, while both groups of isolates were about equally destructive to alfalfa seedlings.

The order of virulence of three isolates on sugar-beet seedlings was retained with seedlings of some additional hosts but was different with others. These same three isolates caused different degrees of stunting of various host plants.

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THE INFLUENCE OF INHERITANCE AND ENVIRONMENT ON THE MILK PRODUCTION AND BUTTERFAT PERCENTAGE OF JERSEY CATTLE[1]

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INTRODUCTION

In the Island of Jersey the importation of cattle has been prohibited since 1763, or for about 40 generations in the life scale of cattle. The Royal Jersey Agricultural Society has systematically tried to standardize the animals by drawing up scales of points and guiding the breeding operations of individual farmers. The Jerseys of North America were imported from this island stock, mostly during the last quarter of the nineteenth century and the present century. The million or so progeny of these imported cattle have been kept separate from other cattle by registration of pure breeding.

From 1900 to 1923 about 14,000 of the half million American Jersey cows[2] were tested for their milk yield over a year's lactation. All of these cows were able to produce the minimum requirement of 360 pounds of butterfat at 5 years of age. Their performance records include the cow's name and number, the name and number of her sire and dam, her age, the length of her record, the amount of milk and butterfat that she produced, the butterfat percentage, and the duration of time she carried her calf. The purpose of the present investigation was so to analyze these records of milk secretion as to ascertain the part played by the inheritance of each ancestor and by the environment in fixing the yield and butterfat percentage of a cow's milk.

The records chosen for study were those of 364, 365, or 366 days. All records were corrected for age.[3] Beyond a correction to the equivalent expected record of 8 years and 3 months, however, no changes in the data were made. While this group represents but a small proportion of the total number, in fact a proportion hardly adequate for a concerted genetic control of the breeding for milk production within this breed as a whole, it does not appear to be heavily selected. The data are, therefore, treated as though selection were absent. The problem with which this paper deals may be illustrated by the pedigrees described below.

PEDIGREES OF HIGH AND LOW MILK PRODUCTION

The sum total of the effects of inheritance or environment on milk secretion may be seen in a typical pedigree of a high milk-producing cow as contrasted with one of low milk production. To make such

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[2] Registry of Merit of Jersey Cattle, American Jersey Cattle Club, New York.

[3] While the actual correction constants were determined specifically for these Jersey cattle data from their own mean age production curves, they are in principle the same as those developed by the writer elsewhere (*12*).[4]

[4] Reference is made by number (italic) to Literature Cited, p. 464.

pedigrees, all the Jersey cows whose mature-form production was under 8,000 pounds were taken as the low-producing group. There were 766 such cows in the 3,753 animals which came from tested dams. For the high-producing group all cows were taken whose production was over 12,000 pounds. There were 738 such cows in the 3,753 animals which came from tested dams. The pedigrees of each cow were run out to the third generation. The average mature-form production of the daughters of each animal in the pedigree was then determined, summed, and averaged. This average appears in the pedigrees presented below. Besides this average, the average production of the dams, the paternal granddams, and the maternal granddams was found and that average also appears in the pedigree.

Low milk yield

- Daughters' milk yields under 8,000 pounds.
  - Sire; daughters' average milk yield 9,376 pounds.
    - Paternal grandsire; daughters' average milk yield 9,844 pounds.
    - Paternal granddam; daughters' average milk yield 10,063 pounds, average paternal granddam's milk yield 10,327 pounds.
  - Dam; daughters' average milk yield 8,639 pounds, average dam's milk yield 8,945 pounds.
    - Maternal grandsire; daughters' average milk yield 9,401 pounds.
    - Maternal granddam; daughters' average milk yield 9,299 pounds, average maternal granddam's milk yield 9,129 pounds.

High milk yield

- Daughters' milk yields above 12,000 pounds.
  - Sire; daughters' average milk yield 10,572 pounds.
    - Paternal grandsire; daughters' average milk yield 10,265 pounds.
    - Paternal granddam; daughters' average milk yield 11,183 pounds, average paternal granddam's milk yield 12,044 pounds.
  - Dam; daughters' average milk yield 11,858 pounds, average dam's milk yield 10,890 pounds.
    - Maternal grandsire; daughters' average milk yield 10,212 pounds.
    - Maternal granddam; daughters' average milk yield 10,710 pounds, average maternal granddam's milk yield 10,062 pounds.

A glance at the pedigree of the average low-producing cow as compared with that of the average high-producing cow shows that throughout the production of the ancestors of the high-producing animals was distinctly greater than that of the low-producing animals. It will be further noticed that on the whole the records of the parents are more nearly like those of their progeny than like those of the grandparents. There is, unfortunately, a selective factor which to some degree impairs this comparison. The cows which were selected for this group of necessity appear in the averages for the daughters of the sire or dam of that group. Similarly, the high-producing cows appear in the averages as daughters of their parents. The effect on the average record of the sires' daughters is not pronounced since

these selected cows are only one-fifth to one-seventh of the whole number of daughters. The effect on the average record for the dams' daughters is quite marked since these selected low- or high-producing daughters are a half of the whole number from which the average is obtained. There appears to be no really satisfactory way to overcome this difficulty. The reader should keep it clearly in mind, however, and realize that the apparent difference in the production of the sire's daughters and dam's daughters has no biological basis but is due only to the particular arrangement of the data. Fortunately this difficulty does not affect the records of the grandparents or the average production of the dams' own records. The marked selection of sires on the basis of the record of their dams is apparent in both pedigrees. The average production of the daughters of all the grandparents is higher for the high-group granddaughters than for the low-group granddaughters. For each given group, the daughters of the paternal and maternal grandsires are about equal in production.

An examination of the actual production of the females of the pedigree shows that the daughters, production resembles that of their dams rather more than that of the grandparents. The prominent selection of the paternal granddams is evident. The maternal granddams are relatively little selected. Their milk yields show a definite similarity to those of the granddaughter, although this similarity is not so great as that of the dams.

Similar pedigrees showing the sum total effect of inheritance and environment on the butterfat percentage of the daughter are presented below. To form the low-testing pedigree 415 Jersey cows were taken that had a butterfat test under 4.8 percent. These cows came from tested dams and were by sires which had tested daughters. This group constituted about the lower sixth of the animals in the Jersey Registry of Merit. The high butterfat testing group consisted of 446 cows having a butterfat test above 5.9 percent. These cows represented the highest testing animals in the Jersey Registry of Merit. They, too, constituted about a sixth of the animals tested and were from tested dams. The pedigrees of these animals were run out to three generations. The average mature-form butterfat percentage of the registry of merit daughters of each individual in these pedigrees was determined. These records were then averaged for each ancestral position in the pedigree. Besides this average, the average butterfat percentage of each ancestral group was determined for the female line of the pedigrees.

Low butterfat percentage

- Daughters' butterfat percentage under 4.9 percent.
  - Sire; daughters' average butterfat percentage 5.18.
    - Paternal grandsire; daughters' average butterfat percentage 5.32.
    - Paternal granddam; daughters' average butterfat percentage 5.26, average paternal granddam's butterfat percentage 5.16.
  - Dam; daughters' average butterfat percentage 4.99, average dam's butterfat percentage 4.96.
    - Maternal grandsire; daughters' average butterfat percentage 5.22.
    - Maternal granddam; daughters' average butterfat percentage 5.12, average maternal granddam's butterfat percentage 5.15.

High butterfat percentage

- Daughters' butterfat percentage above 5.9 percent.
  - Sire; daughters' average butterfat percentage 5.51.
    - Paternal grandsire; daughters' average butterfat percentage 5.41.
    - Paternal granddam; daughters' average butterfat percentage 5.42, average paternal granddam's butterfat percentage 5.49.
  - Dam; daughters' average butterfat percentage 5.75, average dam's butterfat percentage 5.64.
    - Maternal grandsire; daughters' average butterfat percentage 5.42.
    - Maternal granddam; daughters' average butterfat percentage 5.56, average maternal granddam's butterfat percentage 5.55.

These typical pedigrees for cows of low butterfat percentage and for cows of high butterfat percentage display essentially the same general features as those for milk yield. Throughout, the ancestors in the pedigree of the low butterfat tests have lower butterfat-testing daughters than those of the high butterfat tests. Within either group the records of the parents are more nearly like those of their offspring than are the records of the grandparents. The butterfat percentages of the four grandparents are essentially the same within each group. Consequently no selective breeding for butterfat percentage appears to have been practiced in the Jersey breed.

The data for the actual average butterfat percentages of the female ancestors in the pedigree show the importance of the ancestors closest to the animal under study as contrasted with the relative lack of importance of those a generation further removed. The average dams' butterfat percentage of the low-producing line was 4.96 percent, whereas that of the average dam in the high-producing line was 5.64. The grandparents, on the other hand, show for the low-producing line 5.16 and 5.15 percent as their averages, whereas those of the high-producing line were 5.49 and 5.55 percent. From other evidence it has been possible to show that the resemblance one generation further removed (the great grandparents) is even less. The data show that no significant selection of bulls to be used as sires has been made on the basis of their dams' butterfat percentage. The butterfat percentages of the paternal granddams and of the maternal granddams seem for practical purposes to be the same.

The pedigrees here shown graphically illustrate the effects of inheritance and environment on the milk yield or butterfat percentage of particular groups of animals. The problem before us is to analyze these two variables in such a way as to be able to ascribe to each ancestor and to the environment its contribution to the total effect.

The contribution of the particular ancestor to the cow's whole inheritance for milk production is ascertained by comparing the production of the cow with that of her parents, sisters, cousins, and grandparents. The environmental effects are determined by comparing the production of individuals of the same generation within the same herd with that of other generations. The form in which the results are presented is designed to indicate the basic information worthy of consideration in practical breeding as well as the relative importance of the different ancestors in the strict genetic sense.

INFLUENCE OF SIRE ON MILK PRODUCTION AND BUTTERFAT PERCENTAGE

The most frequently used measure of the sire's influence on the milk production of his progeny is the average production of his daughter. This measure is based on the assumption that if milk yield is truly inherited the expectation would be that the sire's daughters would show the type of production which he transmitted to them. His daughters would be expected to resemble each other more than cows of the general breed because of the common inheritance they received from him. If the daughters were full sisters then the resemblance between their productions would be still greater, for here both parents would contribute to the like inheritance. There are 1,830 sires with 2 or more daughters in the Jersey Registry of Merit. The comparison of the milk yields and butterfat percentages of the 110,456 pairs of half sisters sired by these sires gives the data in table 1:

TABLE 1.—*Comparison of milk yield (pounds) and butterfat percentage of 110,456 pairs of half sisters sired by the 1,830 sires with 2 or more daughters in the Jersey Registry of Merit*

Item	Milk-yield data	Butterfat percentage data
Average record	10,065±17	5.37±0.003
Standard deviation	2,470±12	0.515± .003
Coefficient of variation	24.5	9.6
Correlation coefficient	0.24±0.01	0.25± .01

If the average production of these half sisters is compared with that found for the breed as a whole at mature form, 9,849 pounds of milk and 5.31 percent of butterfat, it will be seen that these half sisters are slightly above the breed's average production in milk and practically average in butterfat percentage. The variation in the milk yield is somewhat more than the breed's average (2,254 pounds), while the variation in butterfat percentage is representative (0.516). The correlation coefficients show that there is a fairly well-marked relation between the milk yields or the butterfat percentages of these half sisters. This relation shows the influence of the sire on his daughters' producing capacity since these daughters are from different dams, each dam having a different heredity. The linear equations expressing the relation between the production of one half sister and that of another as determined from the foregoing values are:

Half sister's average milk yield = 7,719 + 0.23 half sister's milk yield

Half sister's average butterfat percentage = 4.03 + 0.25 half sister's butterfat percentage

TABLE 2.—*Average milk yield (pounds) and butterfat percentage of half sisters from a given sire*

Actual milk yield of first daughter	Average milk yield of half sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of half sisters	Actual milk yield of first daughter	Average milk yield of half sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of half sisters
5,000	8,884	3.5	4.90	13,000	10,748	5.5	5.40
7,000	9,350	4.0	5.03	15,000	11,214	6.0	5.53
9,000	9,816	4.5	5.15	17,000	11,680	6.5	5.65
11,000	10,282	5.0	5.28	19,000	12,146	7.0	5.77

Table 2 is based on these equations and illustrates the probable or average production of a cow, half sister to a cow of known production. Thus if the production of the half sister is 7,000 pounds the probable production of another cow by the same sire but by a different dam would be 9,350 pounds. Where one of the sisters has a milk yield of 19,000 pounds, the milk yield of the other tends to be less, 12,146 pounds. The average yields tend to regress toward the average value of the breed when the first half sisters are extremely low or extremely high producers. The same is true for the butterfat percentages.

In breeding practice this type of problem is presented in a variety of ways. It occurs most frequently in a case where the first offspring of a sire has a low record, say 7,000 pounds of milk, and the owner of the sire is considering the advisability of discontinuing his further use. Table 2 shows that a second daughter by this sire would have a probable production of 9,350 pounds of milk. While this is not high, it is better than the 7,000 pounds produced by the first daughter.

The data in table 1 give the information necessary to determine not only the probable production of a bull's daughter, but also the amount of variation which they would be expected to exhibit. The probable production of the bull's daughters determine from that of their half sisters is given in the equation presented above. The amount of variation which the bull's daughters will have around their average production is determined by the correlation coefficient and the standard deviation. This variation is equal to the standard deviation as found in table 1 (2,470 for milk and 0.515 for butterfat percentage) multiplied by the square root of 1 minus the correlation coefficient squared, $S.D.\sqrt{1-r^2}$. The value of the correlation coefficient for the milk yield is 0.24 and for the butterfat percentage 0.25. The standard deviation expected in the bull's daughters' production is therefore 2,397 pounds for milk yield and 0.499 percent for the butterfat percentage. Fifty percent of the daughters would be expected to be within $0.67449 \times 2,397 = 1,618$ pounds on either side of the average production of the group, assuming a normal distribution. Thus if a half sister's production is 9,000 pounds the rest of the bull's daughters would be expected to have an average production of 9,816 pounds. Fifty percent of these daughters would be expected to produce between $9,816 - 1,618 = 8,198$ and $9,816 + 1,618 = 11,434$ pounds of milk. In the same manner it is possible to determine the extreme limits of the production. The limit of the distribution from the average value, 9,816 pounds, to include 99 percent of the daughters is 2.58 times the standard deviation on either side of the mean. In actual figures this limit would be $2,397 \times 2.58$, or 6,192 pounds on either side of the average value. For example, if a bull's first daughter produced 15,000 pounds of milk the expected average production of his other daughters would be 11,214 pounds; 50 percent of the daughters would produce between 9,596 and 12,832 pounds, and 99 percent would produce between 5,022 and 17,406 pounds. These facts bring out a significant point. While it is possible to estimate the average production of these half sisters, there still remains a wide variation within which the individuals half sisters' production may vary, making it impossible to determine the actual production of a single half sister exactly when the only information available is that of a half sister's production.

The degree of resemblance existing between the milk yields or butterfat percentages of half sisters in other breeds of dairy cattle is of some importance since it strengthens any belief that may be held as to the real nature of the facts observed for the cows within the Jersey herd. The correlation between the milk yields of half sisters with a common sire was 0.24 for the Jersey Registry of Merit cows, 0.36 for the advanced registry Holstein-Friesian cows, and 0.13 for the advanced registry Guernsey cattle. For the butterfat percentages the correlations for these Jerseys was 0.25, for the Holstein-Friesian 0.37, and for the Guernsey 0.17 (*11, 13, 14*). The range of values for the different breeds is perhaps greater than would be expected. The results agree, however, in showing that the sire's progeny tend to resemble each other to a noticeable degree, enough so that their records may be used advantageously in pedigree breeding. The average degree of likeness is such as might be expected were the breeding at random so far as the inheritance factors for milk yield or butterfat percentage are concerned and the inheritance by the multiple-factor Mendelian scheme.

Ordinarily the influence of a parent on the progeny is determined by a comparison such as the size of the sire with the size of his daughters. Unfortunately this direct method of comparison is not available in the study of inheritance in a character expressed only by one sex. The correlation may be determined by the following somewhat indirect method. The daughters of a single sire would in any case all fall within an array of a correlation table. In normal correlation the constants of such an array are related to those of the total array in such a manner that the standard deviation of an array within the table is equal to the standard deviation of the whole population multiplied by $\sqrt{1-r^2}$ when r is the correlation sought.

This correlation, while tedious to calculate, makes it possible to determine the relation between two variables, in this case sire and daughter, where only one can be measured. The method is subject to 1 or 2 assumptions and selective factors which should be recognized in the interpretation of the observed facts. In the first place, the correlation surface should be normal. Unfortunately this point cannot be determined, although it is a fair assumption that it is approximately normal since the tables of daughter with dam, etc., are. The array standard deviations are calculated around the rough observational means. If these means do not lie close to a straight line corresponding to the regression line, then the correlation coefficient found by this method is larger than the product moment r. Again, in determining the standard deviation of an array, the number of individuals available materially affects its size. This difficulty may in part, and possibly entirely, be overcome by the use of the corrections of the standard deviations of small samples as worked out by Student (*1*), Fisher (*7*), and others (*2, 30*). The probable errors of the correlation coefficients calculated in this matter are partly dependent on the number of classes and are fairly large. They are not well worked out.

There are 208 sires with 10 or more daughters on which standard deviations have been calculated. In view of the volume of the data it seems inadvisable to list these individually. Each standard deviation was corrected for the influence of the number of daughters as indicated above and the mean obtained, 1,905 pounds for milk yield and

0.443 for butterfat percentage. The standard deviation of the general population is 2,470 pounds for milk and 0.515 for the butterfat percentage. The correlation for milk yield is 0.64 and for butterfat percentage 0.51. On the basis of Mendelian inheritance and random mating these correlation coefficients are somewhat larger than the expected 0.5. However, there are two factors which would tend to make this difference. The ancestry of the daughters on the dam's side is not random, for a fair proportion of the daughters of a sire have common dams as well as sires, which tends to increase the correlation coefficients. The common environment, feeding, care, and management of these cows also serve to make them more alike than they would be solely through inheritance. This factor has a more pronounced effect in these data than it would have in any ordinary correlation table since so much depends on the single variable, daughter's production. We can therefore discount somewhat the numerical value of these correlations between the sire and daughter when making quantitative comparisons with other data. The fact is clear, however, that qualitatively the sire exerts a very decided influence on the quantity of the milk yield and the butterfat percentage of his daughters' daughters.

JOINT INFLUENCE OF SIRE AND DAM ON MILK PRODUCTION AND BUTTERFAT PERCENTAGE

Full sisters with the same sire and dam would be expected to have milk yields and butterfat percentages more nearly alike than would half sisters with only a common sire. The Jersey Registry of Merit cows show just such a difference. There are 1,924 pairs of full sisters in these Jersey records—618 sets of 2, 78 of 3, 15 of 4, and 2 of 5. A comparison of the records of one full sisters with that of another is shown in table 3.

TABLE 3.—*Comparison of milk yield (pounds) and butterfat percentage of 1,924 pairs of full sisters, from the records of the Jersey Registry of Merit*

Item	Milk-yield data	Butterfat percentage data
Average record	10,114±43	5.34±0.01
Standard deviation	2,482±30	0.511± .006
Coefficient of variation	24.6	9.57
Correlation coefficient	0.39±0.01	0.41± .01

The full sisters are essentially the same as the other cows in the Registry of Merit in their mean production of milk and percentage of butterfat. The standard deviation of the milk yields and butterfat percentages, are like those of the other cows. The correlation coefficients show a well-marked relation between the milk yields and butterfat percentages of these full sisters. This relation is much more pronounced than that found for any item of the cow's own conformation correlated with her milk yield or butterfat percentage. It is greater than that for half sisters with a common sire. As the sire's influence was shown by the likeness of the half sister's milk and butterfat production, so the sire's and dam's influence acting jointly is shown by these full-sister records. In view of the fact that the environment of half sisters is like that of full sisters, it follows that this

difference can be accounted for only as the contribution of the heredity of the dam toward making these half sisters alike in their milk secretion. The facts consequently warrant the conclusion that both the sire and dam contribute jointly to the cow's inheritance for milk production and butterfat percentage.

From the foregoing data the relation of the production of full sisters may be put into equation form:

Full sister's milk yield = 6,210 + 0.386 other full sister's milk yield

Full sister's butterfat percentage = 3.18 + 0.405 other full sister's butterfat percentage

As a guide in breeding operations these equations have the same utility as those of the half sisters with a common sire. The joint effect of the sire and dam is strikingly evident in table 4, where the solution of these equations is presented for certain productions. From these equations, the reader can obtain any expected production of full sisters that may be desired. When the production of the first full sister corresponds with any of those given in the table the same answers should be obtained as are given therein. Should the milk yield of a cow be 10,000 pounds the expected milk yield of a full sister would be $10{,}000 \times 0.386 + 6{,}210 = 3{,}860 + 6{,}210 = 10{,}070$ pounds. In like manner the butterfat percentages are also obtained.

TABLE 4.—*Average milk yield (pounds) and butterfat percentage of full sisters*

Actual milk yield of first daughter	Average milk yields of full sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of full sisters	Actual milk yield of first daughter	Average milk yields of full sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of full sisters
5,000	8,140	3.5	4.59	13,000	11,228	5.5	5.40
7,000	8,912	4.0	4.80	15,000	12,000	6.0	5.61
9,000	9,684	4.5	5.00	17,000	12,772	6.5	5.81
11,000	10,456	5.0	5.20	19,000	13,542	5.0	6.01

The variation in the productions of individual cows around that expected of full sisters as seen in table 4 may be determined in the same manner as those derived for the sire. The 50-percent range is found to be $\pm 1{,}531$ pounds for milk yield and ± 0.314 for butterfat percentage. The 99-percent range for milk yield is $\pm 5{,}895$ and for butterfat percentage ± 1.203.

The full-sister correlations for these Jersey cows were 0.39 for milk yield and 0.41 for butterfat percentage. Similar correlations have been determined for the Holstein-Friesian and the Guernsey breeds. These correlations were, for the Holstein-Friesian, 0.55 ± 0.03 for milk yield and 0.46 ± 0.03 for butterfat percentage; for the Guernsey, 0.41 ± 0.02 for milk yield and 0.44 ± 0.02 for butterfat percentage. The data for these three breeds are consequently not very different. Furthermore they are in accord with the hypothesis that the sire and dam are jointly responsible for the milk production and butterfat percentage of their offspring. The data are also in fair accord with the expectation of theoretical inheritance when it is assumed that the character of the milk secreted by a cow is determined by multiple Mendelian factors and that the population is random bred, as such

assumptions lead to an expected correlation between full sisters of 0.5 and between half sisters of 0.25. The actual averages are, for full sisters, 0.44 for milk yield and 0.45 for butterfat percentage, and, for half sisters with a common sire, 0.24 for milk yield and 0.26 for butterfat percentage.

INFLUENCE OF DAM ON MILK PRODUCTION AND BUTTERFAT PERCENTAGE

The influence of the dam on the milk yield of her progeny may be measured in at least two ways—by determining (1) the relation which exists between the records of half sisters when the dam is the common parent, and (2) the correlation between the milk produced by the dam and that produced by her daughter. The data on the milk yield of half sisters with a common dam are presented first.

There are 1,603 of these sisters groups—1,248 sets of 2, 248 of 3, 80 of 4, 17 of 5, 5 of 6, and 5 of 7, making a total of 5,644 pairs. Of this total, 1,924 pairs are full sisters and must be subtracted, leaving 3,720 pairs in all. The constants for measuring the relation between these half sisters are shown in table 5.

TABLE 5.—*Comparison of milk yield (pounds) and butterfat percentage of 3,720 pairs of half sisters with a common dam, from the records of the Jersey Registry of Merit*

Item	Milk-yield data	Butterfat percentage data
Average record	9,882±34	5.36 ±0.01
Standard deviation	2,373±25	.510± .01
Coefficient of variation	23.9	9.5
Correlation coefficient	0.19±0.01	.20 ± .01

The half sisters with a common dam produce slightly less milk than do either the half sisters with a common sire or full sisters. The butterfat percentage of the three groups is practically identical. The variation in milk yield is somewhat less than that for the other two groups. It is, however, in line with the mean production in that it is reduced, thus tending to make the coefficients of variation of the three groups the same. The variation in butterfat percentage is practically identical with that of the other groups both absolutely and in percentage. Characteristically the coefficient of variation in milk yield is two and one-half times as much as that for butterfat percentage, indicating that milk yield is relatively much more variable than is the proportion of its butterfat.

The correlation coefficients are, of course, the most important feature of table 5 from the breeding point of view. The correlations between half sisters of the two kinds (sire or dam in common) are distinctly comparable if not exactly the same. Such being the case it may be concluded that the sire and dam are equally important in determining the quantity of milk produced as well as the percentage of butterfat of their progeny. This conclusion is further borne out by the fact that when the joint influence of the sire and dam is measured the correlations are double those obtained when one is measured separately, as we should expect if the sire and dam contribute equally

to the progeny's genetic constitution for milk production or butterfat percentage.

These facts are of interest from a slightly different point of view, namely, the relative influence of heredity and environment (care, management, etc.) in determining the milk yield or butterfat percentage of the cow. It seems entirely possible that with any such group of cattle as these the milk yield and butterfat percentage of half sisters would be as much influenced by their common environment as those of full sisters. If such is the case, then the difference in the correlations of half sisters and full sisters should measure the extra effect of the common sire or common dam directly attributable to inheritance. The differences between these half and full sisters are on the order of 0.18, which would leave but little remaining as the influence of the common environment. This point is discussed later.

The equations for the relations between the milk yields and butterfat percentages of these half sisters from a common dam are:

$$\text{Half sister's milk yield} = 7{,}955 + 0.195 \text{ other half sister's milk yield}$$

$$\text{Half sister's butterfat percentage} = 4.28 + 0.201 \text{ other half sister's butterfat percentage}$$

Table 6 is based upon these equations, expressing the relation of the half sisters' production for a given production of the cow's first daughter.

TABLE 6.—*Average milk yield (pounds) and butterfat percentage of half sisters from a common dam*

Actual milk yield of first daughter	Average milk yield of half sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of half sisters	Actual milk yield of first daughter	Average milk yield of half sisters	Actual butterfat percentage of first daughter	Average butterfat percentage of half sisters
5,000	8,930	3.5	4.98	13,000	10,490	5.5	5.39
7,000	9,320	4.0	5.08	15,000	10,880	6.0	5.49
9,000	9,710	4.5	5.18	17,000	11,270	6.5	5.59
11,000	10,100	5.0	5.29	19,000	11,660	7.0	5.69

Table 6 shows that half sisters with a common dam resemble each other as closely in their milk yield and butterfat percentage as do half sisters with a common sire. The likeness of either set of half sisters is not so close as that of full sisters. In numerical terms the data show that where one sister has a production of 5,000 pounds her half sister by the same sire would be expected to have a production of 8,884 pounds, her half sister from the same dam 8,930 pounds, and her full sister 8,140 pounds. The half sisters have practically the same expected milk yields. The full sisters are 760 pounds nearer in their production than half sisters with a common sire or half sisters with a common dam. A similar situation exists where the production of the first cow is high. For a cow with a production of 19,000 pounds the half sisters would be expected to produce about 12,000 pounds, the full sisters 13,542 pounds. Again the full sisters tend to be more alike in their production than half sisters. Similar relationships are observed for the butterfat percentage of half and full sisters.

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The standard deviations for the production of the half sisters, when their average record is that indicated by the equation, are 2,298 pounds of milk and 0.5 percent of butterfat. The ranges necessary to include 50 percent and 99 percent of the half sisters on either side of this average value are 1,551 and 5,935 pounds for milk yield and 0.337 and 1.291 for butterfat percentage.

If the three important dairy breeds, Holstein-Friesian, Guernsey, and Jersey, are compared we find the following correlations between the half sisters with common dams: Milk yield Holstein-Friesian 0.38 $\pm$ 0.03, Guernsey 0.15 $\pm$ 0.02, and Jersey 0.19 $\pm$ 0.01; butterfat percentage Holstein-Friesian 0.22 $\pm$ 0.04, Guernsey 0.19 $\pm$ 0.01, and Jersey, 0.20 $\pm$ 0.01. The data for the three breeds are alike in showing a notable resemblance between the production of half sisters with a common dam. Full sisters resemble each other more closely in their milk yield and butterfat percentage than half sisters. The half sisters from a common sire or from a common dam have practically the same degree of resemblance. The hypothesis that the sire and dam are equally and jointly responsible for the milk production and butterfat percentage of their offspring is consequently borne out by this comparison. Furthermore the results are in accord with the expectation of a random-bred Mendelian population. For such a population the relation between full sisters should be measured by a correlation coefficient of 0.5, and between half sisters by a correlation coefficient of 0.25. Actually for the three breeds averaged together the correlation coefficient for the milk yields of full sisters was 0.45 and that for half sisters 0.24. For butterfat percentage the correlation coefficient was 0.43 for full sisters and 0.23 for half sisters.

RELATION BETWEEN MILK YIELD AND BUTTERFAT PERCENTAGE OF DAMS AND DAUGHTERS

Table 7 shows the relation between the milk production and butterfat percentage of daughter and dam. The average milk yield and butterfat percentage of the daughters is given for different groups of dams, progressing from low-producing to high-producing dams. There are 3,753 pairs of these daughter and dam records.

TABLE 7.—*Relation between daughters' and dams' average milk yield (pounds) and butterfat percentage in 3,753 pairs of records*

Number of daughter-dam pairs	Dam's milk yield	Daughter's milk yield	Number of daughter-dam pairs	Dam's butterfat percentage	Daughter's butterfat percentage	Num ber of daugh ter-dam pairs	Dam's milk yield	Daughter's milk yield	Number of daughter-dam pairs	Dam's butterfat percentage	Daughter's butterfat percentage
12	5,500	9,830	2	3.5	4.20	64	15,500	11,330	415	5.7	5.50
200	6,500	8,900	1	3.9	4.70	36	16,500	12,140	300	5.9	5.62
578	7,500	9,240	35	4.1	4.80	15	17,500	11,500	180	6.1	5.64
727	8,500	9,540	89	4.3	4.96	9	18,500	12,060	81	6.3	5.78
772	9,500	9,930	191	4.5	5.02	3	19,500	9,500	48	6.5	5.82
516	10,500	10,300	277	4.7	5.04	3	21,500	17,200	19	6.7	5.86
329	11,500	10,510	442	4.9	5.16				13	6.9	5.84
255	12,500	11,190	545	5.1	5.26				2	7.1	5.20
159	13,500	11,460	604	5.3	5.34				1	7.9	5.70
75	14,500	10,990	508	5.5	5.44						

The average daughters' production for grades of production of the dams show that as the dams' milk production increases the average daughters' production also increases. These increases show some irregularities since the figures presented are the actual average production and not just the trends as given previously. This average production of the daughters as determined from the trend of these milk yields shows that for 1,000 pounds increase in the milk yield of the dam, the daughter's average increases 316 pounds. In other words, the increase of the daughters is about one-third that of the dams.

The results for butterfat percentage are similar. Dams of higher butterfat percentage tend to have daughters that are high in butterfat percentage, and dams whose butterfat percentage is low tend to have daughters whose butterfat percentage is relatively low. Here, again, increase of the butterfat percentage of the dam causes only a fractional increase in the butterfat percentage of the daughter. The average increase of the daughter's butterfat percentage for a 1 percent increase of the dam's is 0.42 percent.

The average milk yield and butterfat percentage of the daughters are both larger than those of their dams. The variation of the milk yields of these daughters is also somewhat greater than that of their dams. The correlation coefficients for the production records of daughter and dam are both significant and of medium value. The constants are given in table 8.

TABLE 8.—*Comparison of milk yield (pounds) and butterfat percentage of daughters and their dams, from the records of the Jersey Registry of Merit*

Item	Milk-yield data		Butterfat percentage data	
	Daughter	Dam	Daughter	Dam
Average record	10,028±27	9,852±25	5.35 ±0.01	5.31 ± 0.01
Standard deviation	2,420±19	2,285±18	.513± .004	.518± .004
Coefficient of variation	24.1	23.2	9.6	9.8
Correlation coefficient	0.30±0.01		0.42±0.01	

The fact that the daughters' production is somewhat higher than that of their dams is a matter of some moment if it truly represents an increase in yield due to better heredity of the daughters or to a better understanding of nutrition. Such a change would indicate real progress. Unfortunately the differences cannot be directly attributed to these causes, for the increase in production is also subject to other interpretations. The animals tested for Registry of Merit might come from a more highly culled class than their dams, a culling based on previous production records. Such a culling would account for the small difference observed.

The relation between the milk yields and the butterfat percentages of daughters and dams is shown graphically in figures 1 and 2. The irregular line indicates the actual average production of daughters for a given production of dams as shown in table 7. The straight lines represent the average trend of the data as derived from the regression equations.

Daughters' milk yield = 6,920 + 0.32 dams' milk yield

Daughters' butterfat percentage = 3.12 + 0.42 dams' butterfat percentage

The standard deviations and coefficients of variation of milk yield and butterfat percentage are well within the range of those ordinarily found for dairy cattle. The variation of the butterfat percentage is proportionately only two-fifths that of the milk yield.

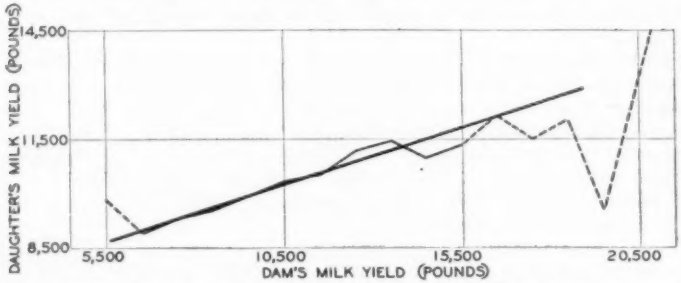

FIGURE 1.—Relation between the milk yields of daughter and dam. Straight line represents average trend of the data; solid line connects averages established by large volume of data and broken line those established by few data.

The correlation coefficients for milk yield and butterfat percentage of daughter and dam show that inheritance plays an important part in milk secretion. The degree of relationship is lower for milk yield than for butterfat percentage as determined for these data. A comparison of these results with those from other breeds shows that such a

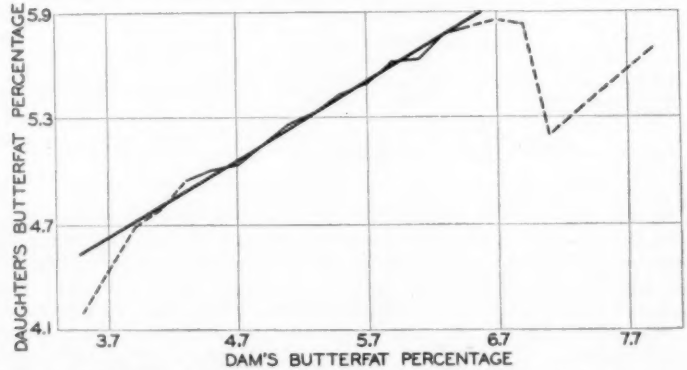

FIGURE 2.—Relation between the butterfat percentages of daughter and dam. Straight line represents general trend of the data; solid line connects averages established by large volume of data and broken line those established by few data.

difference does not always occur. The correlation coefficients for the milk yield and butterfat percentage of Holstein-Friesian cattle are 0.50 ± 0.02 and 0.41 ± 0.02, respectively. The difference is in a direction opposite to that found for the Jerseys. The relation between the milk yield and butterfat percentage of daughter and dam in the Guernseys is in the same direction as that for the Jerseys, 0.36 ± 0.02 for milk yield and 0.42 ± 0.02 for butterfat percentage.

The Jersey Registry of Merit cows show a pronounced negative correlation between the milk yield of the cow and the butterfat percentage of her milk, the larger the milk yield the lower the butterfat test of the milk. This correlation is equal to -0.29 ± 0.01 for the daughters and -0.27 ± 0.01 for the dams. A slight negative correlation would therefore be expected between the production of the daughters and the butterfat percentage of the dams brought about by this physiological relationship. Such zero-order correlations are actually found; daughter's milk yield with dam's butterfat percentage equals -0.12 ± 0.01 and daughter's butterfat percentage with dam's milk yield equals -0.11 ± 0.01. If these correlations are determined with the proper daughter's and dam's production or butterfat percentage held constant, the correlations are less in value. The correlation between daughter's milk yield and dam's butterfat percentage for a constant butterfat percentage of the daughter and a constant milk production of the dam equals 0.08 ± 0.02, and the correlation between the daughter's butterfat percentage and the dam's milk yield for a constant daughter's milk yield and dam's butterfat percentage equals 0.08 ± 0.02, values which are scarcely significant. The results are thus comparable with those of the Holstein-Friesian breed.

RELATION BETWEEN MILK YIELD AND BUTTERFAT PERCENTAGE OF GRANDPARENTS AND GRANDDAUGHTERS

Two individuals may have none, any, or all of their grandparents in common. In 1 combination they may have all grandparents in common; in 4 combinations they may have 3 grandparents in common; in 6 combinations, 2, in 4 combinations 1, and in 1 combination none—16 cousin combinations in all. Within a single class the degree of genetic correlation will also vary. Those individuals with 4 common grandparents may have these as the result of a repeated mating of the same sire and dam, when they will be full sisters, matings of the same sire to full sisters, matings of full brothers to the same cow, or matings of full brothers and sisters. On the basis of multiple-factor inheritance for a character and random segregation, the expected genetic correlations for such individuals are distinctly different.

There are equally significant differences in the other ancestral groups. So far as possible an attempt is made to separate these genetically different groups. The data of table 9 show the correlations between the production of the granddaughters for the different combinations of common grandparents. The paternal grandsire is represented by A, the paternal granddam by B, the maternal grandsire by C, and the material granddam by D. The first column of table 9 shows the number of common grandparents for the particular cows whose milk yield or butterfat percentage is correlated. The records are presented for individuals having 4, 3, 2, and 1 common grandparent. The other possible combinations of grandparents had so few pairs of granddaughters for comparison that they are omitted from the table. The A C D group, for example, had but 66, the B C D but 60, etc.

TABLE 9.—*Correlation between the milk yield (pounds) or butterfat percentage of individual cows having the indicated relationship*

Grandparents common [1]	Parents common	Number of pairs	Correlation coefficients		Mean		Standard deviation		Prediction equations [2]		Variation range to include—			
											50 percent		99 percent	
			Milk yield	Butterfat percentage	Milk yield	Butterfat percentage	Milk yield	Butterfat percentage	Milk yield	Butterfat percentage	Milk yield	Butterfat percentage	Milk yield	Butterfat percentage
A B C D	Sire and dam	1,924	0.39	0.41	10,114	5.34	2,482	0.51	6,170+0.39 M	3.15+0.41 F	1,541	0.31	5,895	1.20
A B C D		474	.26	.28	9,846	5.38	2,450	.51	7,286+ .26 M	3.87+ .28 F	1,596	.33	6,104	1.26
A B C	Sire	14,426	.31	.29	10,161	5.38	2,676	.51	7,011+ .31 M	3.82+ .29 F	1,716	.33	6,564	1.26
A B D	do	720	.30	.24	10,727	5.42	2,286	.55	7,509+ .30 M	4.12+ .24 F	1,471	.36	5,627	1.38
A B	do	99,458	.24	.25	10,057	5.37	2,463	.51	7,643+ .24 M	4.03+ .25 F	1,613	.33	6,169	1.27
A and C are same bull		4,612	.25	.27	10,128	5.38	2,787	.37	7,596+ .25 M	3.93+ .27 F	1,820	.24	6,963	.92
A C		1,374	.12	.24	9,651	5.48	2,145	.48	8,493+ .12 M	4.16+ .24 F	1,437	.31	5,495	1.20
B C		1,566	.15	.10	10,024	5.38	2,579	.50	8,520+ .15 M	4.84+ .10 F	1,720	.34	6,579	1.28
C D		948	.35	.14	11,235	5.43	2,870	.41	7,303+ .35 M	4.67+ .14 F	1,813	.27	6,935	1.05
C D	Dam	3,720	.19	.20	9,882	5.36	2,373	.51	8,004+ .19 M	4.29+ .20 F	1,572	.34	6,011	1.29
A		1,656	.23	.22	9,794	5.39	2,393	.50	7,541+ .23 M	4.20+ .22 F	1,571	.33	6,009	1.26
B		489,526	.02	.07	9,750	5.34	2,322	.52	9,555+ .02 M	4.97+ .07 F	1,566	.35	5,991	1.34
C		26,028	.06	.07	10,414	5.34	2,464	.58	9,789+ .06 M	4.97+ .07 F	1,659	.39	6,347	1.49
D		64,150	.12	.04	9,827	5.36	2,386	.50	8,648+ .12 M	5.15+ .04 F	1,598	.34	6,112	1.29
		3,198	.15	.04	9,773	5.37	2,373	.54	8,307+ .15 M	5.16+ .04 F	1,582	.36	6,053	1.39

[1] A = paternal grandsire; B = paternal granddam; C = maternal grandsire; and D = maternal granddam.
[2] M or F = Milk yield or butterfat percentage of cow having indicated relationship to the first.

The comparison of milk production or butterfat percentage of the pairs of granddaughters shows the largest correlations for the cows that have the largest number of grandparents in common and progressively smaller correlations as the number of common grandparents diminishes. When the cows have four grandparents in common because of having had the same sire and dam their milk yields or butterfat percentages are most nearly alike, as indicated by the correlations 0.39 and 0.41. The next highest correlations are found where the cows have three grandparents in common, the paternal grandsire and granddam being common to both because the sire was the same bull. Following these in the degree of their relationship are the cows which have all four grandparents in common, the sires of these cows being brothers and the dams sisters. The cows which have but two grandparents in common show a still smaller correlation between milk yields or butterfat percentages. Cows with but one common grandparent show the smallest relation between their productions. The results of this study are consequently what would be expected for an inherited character, namely, that the greater the number of common ancestors the greater the degree of resemblance between the related individuals.

The number of pairs having the common ancestors shown in table 9 is enlightening. There are 609,676 possible pairs of cows in these data having a common paternal grandsire, A; 144,052 pairs having a common paternal granddam, B; 89,764 cows having a common maternal grandsire, C; and 11,374 having a common maternal granddam, D. For the first generation there are 112,390 pairs of cows with common sires to 5,644 with common dams. The notably greater distribution of the sire's germ plasm is evident. Of the 609,676 pairs with common paternal grandsires a third had other common grandparents. Of the 144,052 pairs with B in common, 82 percent had other common grandparents. In the maternal line 29 percent of the cows having a common grandsire had other common grandparents, while of the cows with common granddams 72 percent had other common grandparents. Some combinations of grandparents are quite rare. It is unlikely that two cows would have A C D, B C D, A D, or B D in common without having other common grandparents. This uneven distribution of grandparents becomes significant in considering ancestral correlation for milk yield or butterfat percentage, especially when these are obtained by indirect means (ratio of standard deviation of daughters of a single sire to the standard deviation of the whole population), since the chance of the cows having other ancestors repeated besides the sire in question is great. The means and standard deviations of the different ancestral groups vary somewhat. The variation does not appear to be excessive, however.

PATERNAL GRANDDAM

The effect of the inheritance received from the granddam on the granddaughter's milk secretion may be measured directly by comparing the amount of milk secreted by each pair of granddams and granddaughters. There are 2,709 pairs of paternal granddams and granddaughters for comparison. The data showing the average granddaughters' records for given productions of the paternal granddams are presented in table 10.

TABLE 10.—*Relation between granddaughters' and paternal granddams' milk yield (pounds) and butterfat percentage in 2,709 pairs of records*

Pairs of granddaughter-granddam (number)	Paternal granddam's milk yield	Granddaughter's milk yield	Pairs of granddaughter-granddam (number)	Paternal granddam's butterfat percentage	Granddaughter's butterfat percentage	Pairs of granddaughter-granddam (number)	Paternal granddam's milk yield	Granddaughter's milk yield	Pairs of granddaughter-granddam (number)	Paternal granddam's butterfat percentage	Granddaughter's butterfat percentage
3	5,500	9,500	10	4.1	5.16	157	13,500	11,017	448	5.7	5.44
88	6,500	9,300	87	4.3	5.12	71	14,500	10,424	195	5.9	5.42
252	7,500	9,130	169	4.5	5.06	94	15,500	11,195	122	6.1	5.48
458	8,500	9,479	118	4.7	5.32	82	16,500	11,019	27	6.3	5.56
311	9,500	9,701	372	4.9	5.28	182	17,500	10,815	34	6.5	5.46
433	10,500	10,504	379	5.1	5.28	20	18,500	9,750	19	6.7	5.49
363	11,500	10,173	305	5.3	5.34	28	19,500	10,640	2	6.9	5.91
167	12,500	9,693	422	5.5	5.46						

The general trend of the granddaughters' production in either milk or butterfat percentage for increasing production of the granddams is

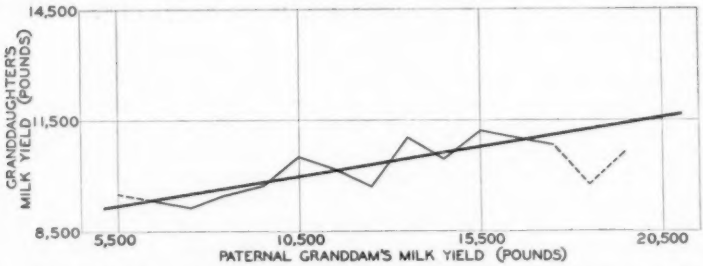

FIGURE 3.—Relation between the milk yields of granddaughters and paternal granddams. Straight line represents average trend of the data; solid line connects averages established by large volume of data and broken line those established by few data.

toward somewhat larger values. For 1,000 pounds increase in the paternal granddams' milk yield, the granddaughters' milk yield increases 159 pounds. The increase of the granddaughters is conse-

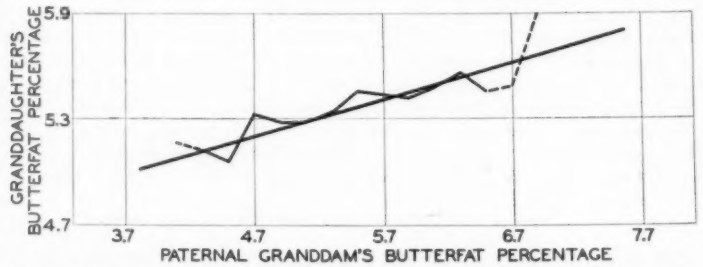

FIGURE 4.—Relation between the butterfat percentages of granddaughters and paternal granddams. Straight line represents average trend of the data; solid line connects averages established by large volume of data and broken line those established by few data.

quently about one-sixth that of the granddams. The results for butterfat percentage are similar. In general paternal granddams whose butterfat percentage is high have granddaughters with some-

what higher butterfat tests than do granddams whose butterfat percentage is low. On an average when there is a 1-percent difference in the butterfat percentage of the granddams, the average tests of the granddaughters differ by 0.21 percent. These results are shown in figures 3 and 4. The constants to measure these are shown in table 11.

TABLE 11.—*Comparison of milk yield (pounds) and butterfat percentage of granddaughters and paternal granddams, from the records of the Jersey Registry of Merit*

Item	Milk-yield data		Butterfat percentage data	
	Granddaughter	Granddam	Granddaughter	Granddam
Average record	10,057±31	11,146±40	5.35 ±0.01	5.32 ±0.01
Standard deviation	2,430±22	3,105±28	.509± .005	.501± .005
Coefficient of variation	24.2	27.9	9.5	9.4
Correlation coefficient	0.20±0.01		0.21±0.01	

The supposed changes of domestic breeds of animals since they separated from their wild progenitors have furnished presumptive evidence for the theory of evolution. The presumed changes in the milk yields of dairy cattle have played their part in this argument, a part possibly greater than the facts warrant, for records of production adequate enough for any real selection are a recent acquisition to animal husbandry. It is questionable if prior to our own generation there was any selection of dairy stock for breeding which was concerted enough to be in any sense effective. The production of the paternal granddams when compared with that of the whole Jersey breed and with that of the maternal granddams indicates that selection for larger milk secretion is in progress in the Jerseys. The average milk yield of these paternal granddams is about 1,100 pounds more, that is, one-tenth more than for the average cow in the advanced registry. The evidence thus shows that there has been a real selection of bulls used for breeding on the basis of the milk-production record of their dams. No such selection has been practiced for the butterfat percentage, however, the average butterfat record of the paternal granddam being no higher than that of the breed average. The selective breeding that is actually practiced emphasizes the milk yield. The degree of this selection within the Jersey Registry of Merit animals is greater than it is with a sister breed, the Holstein-Friesian, as shown by similar studies of this breed.

The foregoing data arranged to give the granddaughters' probable milk secretion when they have paternal granddams of known production is:

Granddaughter's milk yield = 8,286 + 0.16 paternal granddam's milk yield

Granddaughter's butterfat percentage = 4.21 + 0.21 paternal granddam's butterfat percentage

These equations give the mean production of the granddaughters. The variation on either side of this mean necessary to include 50 percent of the granddaughters from granddams of the same yield is 1,619 pounds for the milk yield and 0.34 for the butterfat percentage. To

include 99 percent of the granddaughters, the range is 6,185 pounds for the milk production and 1.30 for the butterfat percentage.

MATERNAL GRANDDAM

The production records of 1,466 granddaughters and the corresponding record for the maternal granddams are shown in table 12. There is a definite increase in milk secretion by the granddaughters as the milk secreted by the maternal granddam increases. This increase is somewhat irregular but the trend is evident. For each 1,000 pounds increase in the granddams' production of milk the granddaughters show an average rise of 155 pounds, or about one-sixth of the granddams' increase. For the butterfat percentage an increase of 1 percent for the granddams results in an increase of 0.26 percent in the average granddaughters' butterfat percentage.

TABLE 12.—*Relation between granddaughters' and maternal granddams' milk yield (pounds) and butterfat percentage in 1,466 pairs of records*

Granddaughter-granddam pairs (number)	Maternal granddam's milk yield	Granddaughter's average milk yield	Granddaughter-granddam pairs (number)	Maternal granddam's butterfat percentage	Granddaughter's average butterfat percentage	Granddaughter-granddam pairs (number)	Maternal granddam's milk yield	Granddaughter's average milk yield	Granddaughter-granddam pairs (number)	Maternal granddam's butterfat percentage	Granddaughter's average butterfat percentage
109	[1] 7,000	9,210	8	[1] 4.2	4.82	50	13,500	10,980	203	5.5	5.43
239	7,500	9,990	27	4.3	5.12	24	14,500	10,540	198	5.7	5.44
302	8,500	9,800	80	4.5	5.24	12	15,500	11,000	123	5.9	5.53
299	9,500	10,310	107	4.7	5.17	7	16,500	11,070	76	6.1	5.60
208	10,500	10,330	166	4.9	5.26	15	17,500	10,570	32	6.3	5.56
128	11,500	10,380	167	5.1	5.32	6	[2] 18,000	12,500	33	[2] 6.4	5.70
67	12,500	10,250	246	5.3	5.36						

[1] Below. [2] Above.

The average milk yields and butterfat percentages of the granddaughters are somewhat larger than those of the maternal granddams. The averages and constants of variation for the 1,466 pairs are given in table 13.

TABLE 13.—*Comparison of milk yields (pounds) and butterfat percentage of granddaughters and maternal granddams, from the records of the Jersey Registry of Merit*

Item	Milk-yield data		Butterfat percentage data	
	Granddaughter	Granddam	Granddaughter	Granddam
Average record	10,125±45	9,623±39	5.37 ±0.01	5.35 ±0.01
Standard deviation	2,549±32	2,236±28	.517± .006	.513± .006
Coefficient of variation	25.2	23.2	9.6	9.6
Correlation coefficient	0.136±0.017		0.254±0.016	

The average milk production of the paternal granddams was more than 1,100 pounds greater than the average of all cows that had been tested. The evidence was consequently in line with the belief that the bulls used as breeders came from dams that produced more than the average amount of milk. Such a selection for all animals within

the ancestry could conceivably affect the production of milk. It is a significant fact that the average production of the maternal granddams, 9,623 pounds, is, if anything, below average rather than above it. These cows are probably not so far below the breed average as would appear from the figures, for an increase in recorded production has been going on within the Jersey Registry of Merit for some time, owing possibly to better breeding or perhaps to a better understanding of those factors that make for a high test record and to a more stringent selection of the animals that enter the test. Such an increase would make the lower producing maternal granddams, since they would be one decade removed, really average-producing cows for the breed. The conclusion is therefore warranted that the cows selected for paternal granddams are a highly selected group while the maternal granddams are not more than average cows. As the data on the inheritance of production all point to the fact that the sire's side and the dam's side of the pedigree are equally important in determining the milk production of their progeny, and as the maternal granddams are no better than breed average in production,

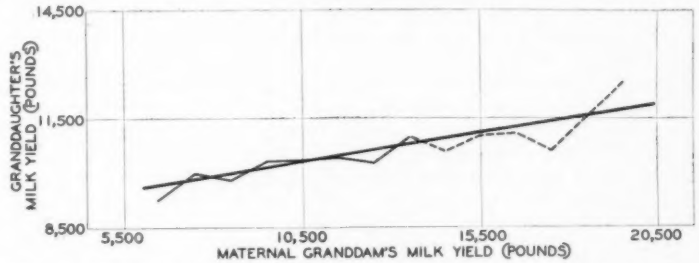

FIGURE 5.—Relation between the milk yields of granddaughters and maternal granddams. Straight line represents average trend of data; solid line connects averages established by large volume of data and broken line those established by few data.

it is apparent that no selection of the higher producing cows for breeders has been practiced. Good breeding practice calls for a selection of better producing maternal granddams. The butterfat percentages of the granddaughters and the granddams are essentially the same. Furthermore they are practically the same as those found for the average of all the advanced registry. These facts point to the conclusion that the breeding of Jersey cows for butterfat percentage is practically at random.

The average production of the granddaughters for a given production of the maternal granddams is shown graphically in figure 5 for milk yield and in figure 6 for butterfat percentage. The straight lines which represent the average trend of the data are derived from the following equations:

Granddaughter's milk yield = 8633 + 0.16 maternal granddam's milk yield

Granddaughter's butterfat percentage = 4.00 + 0.26 maternal granddam's butterfat percentage

These equations, like others presented, simply represent average trends. The variation necessary to include 50 percent of the daugh-

ters around this average is 1,722 pounds of milk and 0.35 percent of butterfat on either side of this average value. The range necessary to include 99 percent of the granddaughters having maternal granddams of a single grade of production is 6,539 pounds of milk or 1.33 percent of butterfat on either side of this production.

The relative contributions of the paternal and maternal granddams to the productivity of the granddaughter are important. A comparison of the records of maternal granddams found in this section with those shown in the section on paternal granddams gives the impression that the effects of these two grandparents are essentially alike. The coefficients of correlation for a grandparent and the grandchild for any Mendelian character dependent on multiple factors for its expression would be expected to equal 0.25 if the breeding has been at random within the group of animals studied. The data just presented show that there has been but little selective breeding within the Jerseys so far as the maternal granddams are concerned. The correlation coefficient between the maternal granddam's milk yield and that of the granddaughter is consequently only about half the value that would be expected, 0.136 to 0.25. The value of the

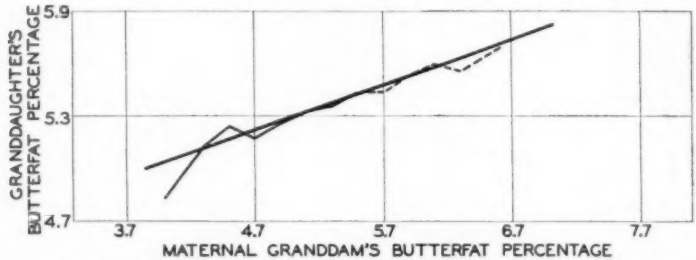

FIGURE 6.—Relation between the butter-fat percentages of granddaughters and maternal granddams. Straight line represents average trend of data; solid line connects average established by large volume of data and broken line those established by few data.

correlation coefficient for the butterfat percentages, 0.254, is as expected.

The correlation coefficients for the paternal granddams' and granddaughters' milk yields and butterfat percentages were, respectively, 0.2 and 0.21. The paternal granddams showed a slightly larger correlation coefficient for milk yield and a slightly smaller coefficient for butterfat percentage than did the maternal granddam. The results throughout are of the same general order of magnitude. From these results it may be concluded that the influence of the paternal and maternal granddams on the granddaughters' milk yield and butterfat percentage is of nearly equal value; in other words, the contribution of these animals to the granddaughters' inheritance is practically the same. This conclusion is strengthened by a comparison of the results from the three breeds, Holstein-Friesian, Guernsey, and Jersey. In all cases the two types of grandparents are about equally important. In terms of correlation these relations for granddaughters' and granddams' records are, for milk yield: Paternal granddam, Holstein-Friesian, 0.26 ± 0.04; Guernsey, 0.16 ± 0.07; and Jersey, 0.20 ± 0.01; for the maternal granddam, Holstein-Friesian, 0.31 ± 0.05; Guernsey, 0.20 ± 0.02; and Jersey,

0.14 ± 0.02. Similar results were obtained from a study of the butterfat percentages. For the Holstein-Friesian, Guernsey, and Jersey breeds, the butterfat percentages of the paternal granddams are correlated with those of their granddaughters to the following extent: 0.09 ± 0.04, 0.15 ± 0.07, and 0.21 ± 0.01. The maternal granddams' butterfat percentages are correlated with their granddaughters' as follows: Holstein-Friesian, 0.19 ± 0.05; Guernsey, 0.20 ± 0.02; and Jersey, 0.25 ± 0.02. The average correlations of the grandparents with those of the granddaughters are, for milk yield, 0.21, and for butterfat percentage, 0.18.

If these average correlations for the grandparents are compared with the average correlation of the daughters and their dams as given earlier, 0.45 for milk yield and 0.42 for butterfat percentage, they are seen to be nearly half the value. This is as it should be if the inheritance of the characters milk yield and fat concentration in the milk are dependent upon multiple Mendelian factors assorting at random.

ASSORTIVE MATING

With milk and its products occupying such an important place in the economic structure of our agriculture, it would seem that there would be a definite tendency on the part of breeders of dairy cattle to mate like individuals. The actual amount of such assortive mating has an interest to all students of breeding because it measures the effective selection and breeding which is taking place within dairy cattle for a given character. If selection with assortive mating is to be a real force in molding the characteristics of the breed, several points must be kept in mind. First and foremost, the sires to be used in the different herds must, in large part, come from superior parents. As already shown, many of the Jersey sires meet this qualification. Such sires should then have the best mates which the breed affords. Some measure of the amount of this selective breeding may be had by relating the milk yield of the sire's dam with the milk yield of the cows to which he is bred. This information is given in table 14.

TABLE 14.—*Relation between dams' and paternal granddams' milk yield (pounds) and butterfat percentage in 1,375 pairs of records*

Dam-paternal granddam pairs (number)	Paternal granddam's milk yield	Dam's average milk yield	Dam-paternal granddam pairs (number)	Paternal granddam's milk yield	Dam's butterfat percentage	Dam-paternal granddam pairs (number)	Paternal granddam's milk yield	Dam's average milk yield	Dam-paternal granddam pairs (number)	Paternal granddam's milk yield	Dam's butterfat percentage
45	[1] 7,000	8,830	51	[1] 4.4	5.39	82	13,500	10,670	262	5.7	5.39
103	7,500	9,520	84	4.5	5.22	37	14,500	9,580	83	5.9	5.42
246	8,500	9,440	62	4.7	5.37	65	15,500	10,810	61	6.1	5.42
131	9,500	9,770	193	4.9	5.30	42	16,500	10,600	9	6.3	5.59
210	10,500	10,110	188	5.1	5.39	138	17,500	10,790	15	6.5	5.33
165	11,500	10,140	160	5.3	5.38	30	[2] 18,000	11,200	10	6.7	5.36
81	12,500	10,230	197	5.5	5.33						

[1] Below [2] Above.

There are 1,375 sires whose dams' production is known bred to cows whose own production records are known. In the terminology of this study, since it is based on the daughters, these ancestors have the relation paternal granddam to dam in the pedigrees. The data of table 14 show that there is a fair relation between the production of the paternal granddams and that of the dams. Sires with dams having larger milk yields tend to be bred to cows which are themselves better than average in production. For every 1,000 pounds of milk increase in the paternal granddam's production, there is an increase of 140 pounds in the production of the dams.

The facts are different for the butterfat percentages. There is practically no relation between the paternal granddams' butterfat percentages and those of the dams.

The average milk yield and butterfat percentage of these two groups, together with their variations and correlations are shown in table 15.

TABLE 15.—*Comparison of milk yield (pounds) and butterfat percentage of paternal granddams and dams, from the records of the Jersey Registry of Merit*

Item	Milk yield data		Butterfat percentage data	
	Granddam	Dam	Granddam	Dam
Average record	11,526±61	10,042±45	5.31±0.01	5.36±0.01
Standard deviation	3,336±43	2,491±32	.49± .01	.49± .01
Coefficient of variation	28.9	24.8	9.2	9.1
Correlation coefficient	0.20±0.02		0.06±0.02	

As indicated earlier, the milk yield of the paternal granddams is considerably larger than that of the rest of the breed. Their butterfat percentages are but average, however. The dams' production and butterfat percentage are comparable to those of the rest of the breed. The correlation coefficient for the milk yields of these two ancestors is 0.20 ± 0.02 and for the butterfat percentages, 0.06 ± 0.03. Consequently the data show a little assortive mating for milk yield but none of any consequence for butterfat percentage. The correlations for these ancestors in the Jersey pedigrees are just a little higher than those for other breeds. The correlations for the paternal granddams and dams are: Holstein-Friesian, 0.14 ± 0.06 for milk yield and 0.001 ± 0.061 for butterfat percentage; and Guernsey, 0.13 ± 0.02 for milk yield and 0.01 ± 0.02 for butterfat percentage. The probable errors show that the values are comparable.

Another measure of assortive mating as practiced in Jersey cattle breeding is the relation between the records of the paternal and maternal granddams. The average milk yields and butterfat percentages of the maternal granddams for given yields of the paternal granddams are seen in table 16. There are 1,146 pairs of paternal and maternal granddams in the pedigrees of these Jersey cattle. A slight trend is noted in the relation of the milk yields, but there is no particular change in the butterfat percentages of these grandparents. The data showing the variations and correlations of the two groups are given in table 17.

TABLE 16.—*Relation between the paternal and maternal granddams' milk yield (pounds) and butterfat percentages in 1,146 pairs of records*

Paternal and maternal granddam, pairs (number)	Paternal granddam's milk yield	Maternal granddam's milk yield	Paternal and maternal granddam, pairs (number)	Paternal granddam's butter fat percentage	Maternal granddam's butter fat percentage
18	[1] 7,000	8,280	21	[1] 4.4	5.05
24	7,500	9,170	28	4.5	5.37
99	8,500	9,560	27	4.7	5.22
40	9,500	9,250	57	4.9	5.37
81	10,500	10,120	65	5.1	5.35
63	11,500	10,010	57	5.3	5.41
34	12,500	9,740	75	5.5	5.37
27	13,500	10,200	128	5.7	5.38
11	14,500	10,680	29	5.9	5.60
25	15,500	10,660	21	6.1	5.44
14	16,500	10,140	14	[2] 6.2	5.36
75	17,500	9,850			
11	18,000	10,230			

[1] Below. [2] Above.

The selection of the sires on the basis of the record of their dams is again evident in these data; these cows producing 2,000 pounds of milk more than the dams of the cows to which the sires will be bred. These cows are not selected for their butterfat percentage. The standard deviation of the paternal granddams' milk yield has likewise markedly increased, the increase being correspondingly greater than the average milk yield, as shown by the larger coefficient of variation. The correlation coefficient for the milk yields of these granddams is statistically significant, showing that there is some assortive mating.

TABLE 17.—*Comparison of milk yield (pounds) and butterfat percentage of paternal and maternal granddams, from the records of the Jersey Registry of Merit*

Item	Milk-yield data		Butter at percentage data	
	Paternal granddam	Maternal granddam	Paternal granddam	Maternal granddam
Average record	11,847±102	9,807±70	5.34±0.01	5.37±0.01
Standard deviation	3,464± 72	2,366±49	.50± .01	.46± .01
Coefficient of variation	29.2	24.1	9.4	8.6
Correlation coefficient	0.12±0.03		0.13±0.03	

The degree of assortive mating in Jersey breeding as it is now practiced is essentially the same as that noted in the Holstein-Friesian and Guernsey breeds. The relations between the milk production and butterfat percentage of the dams and paternal granddams in these two breeds were found to be essentially like those given above, viz, 0.14 ± 0.06 and 0.13 ± 0.02 for milk yield and 0.00 ± 0.06 and 0.01 ± 0.02 for butterfat percentage. The similarity is also carried to the paternal and maternal granddam relations, the correlations being, for Holstein-Friesian, 0.30 ± 0.09 for milk yield and 0.04 ± 0.09 for the butterfat percentage, and Guernseys 0.10 ± 0.03 for milk yield and 0.12 ± 0.03 for butterfat percentage.

These assortive mating coefficients are all low. They are comparable to those recorded by Pearson and Lee (*18*) for stature, span, and forearm length in man. They are somewhat lower than those noted for weight, height at withers, depth at withers, heart girth, and paunch girth in a similar group of cows in this same Jersey breed, and they are much lower than those noted for the width at hips, body length, and rump length of Jersey cattle (*16*). The Jersey cattle, and quite possibly other breeds, are in the anomalous position of having the emphasis placed on their conformational points, the fancy points, rather than on the milk yield or butterfat percentage, the economic points in their breeding.

RELATIVE INFLUENCE OF HEREDITY AND ENVIRONMENT ON MILK YIELD AND BUTTERFAT PERCENTAGE

The correlation coefficients for the different ancestral combinations furnish the material from which an estimate may be obtained of the relative importance of the effects of heredity and environment on milk production and butterfat percentage. Since the parents of a cow are reared to maturity some years before the animal's own growth period it is evident that the environment of the parent and offspring are likely to be quite different. Full and half sisters, on the other hand, are likely to be raised together at the same period of their development. Such being the case, the environmental influences of similar feeding, care, etc., would tend to make them alike. The effect of the similarity in conditions surrounding these sisters is represented in Wright's (*25*, *26*, *27*, *28*, *29*) terminology by e^2. The effect of heredity he calls h^2, and the determination of heredity by dominance deviation d^2, in the correlation between genotypes of different mates. The effects of the common environment and of dominance could theoretically be combined in all gradations. The analysis of the limiting cases (1) assuming dominance to be nonexistent, and (2) assuming the environmental effect to be absent is much simpler, however. By comparing the results of these analyses it is possible to determine the effect of each variable on the milk secretion and butterfat percentage of the Jersey cow. The correlations presented lead to the following equations:

Assortive mating is somatic and genetic factors are without dominance:

$$r_{sd}=\frac{m}{h^2} \qquad r_{so}=r_{do}=1/2h^2(1+r_{sd})$$

$$r_{oo}=1/2h^2(1+m)+e^2 \qquad r_{oo}=1/4h^2(1+2m+m^1)+e_1^2, \tag{1}$$

where s = sire, d = dam, o = daughter, oo = sister, and oo^1 = half sister.

Assortive mating as above but with genetic factors showing dominance and no effect of environment:

$$r_{sd}=\frac{m}{h^2(1-d^2)} \qquad r_{so}=r_{do}=1/2h^2(1-d^2)(1+r_{sd})$$

$$r_{oo}=1/2h^2\left(1-\frac{d^2}{2}\right)(1+m) \qquad r_{oo}{}^1 1/4h^2(1-d^2)(1+2m+m^1) \tag{2}$$

Assortive mating is genetic and no dominance of factors, environment as before:

$$r_{sd}=h^2m \qquad r_{so}=r_{do}=1/2h^2(1+m)$$
$$r_{oo}=1/2h^2(1+m)+e_1^2 \qquad r_{oo}{}^1=1/4h^2(1+2m+m^1)+e_1^2 \tag{3}$$

The numerical data necessary to complete this analysis as taken from the material previously presented are as follows: Correlation coefficients for the relation of the milk yields or butterfat percentages of half sisters with a common sire 0.24 and 0.25, sisters 0.39 and 0.41, half sisters with a common dam 0.19 and 0.2, daughter and dam 0.3 and 0.42. The assortive mating correlation between the sire and dam remains to be determined. The fact that the correlations between parent and offspring are 0.3 and 0.42, those between the paternal granddam and dam are 0.2 and 0.06, and those between the paternal and maternal granddams are 0.12 and 0.13, shows that the assortive mating correlations between the sire and dam are approximately 0.07 for milk yield and 0.01 for butterfat percentage. These correlations give the following values (table 18) for measuring the effect of dominance (d^2) and of variations in heredity (h^2) and environment (e^2), etc., on the Jersey cow's milk secretion and butterfat percentage.

TABLE 18.—*Values for measuring the effect of dominance and of variations in heredity and environment, etc., on milk secretion and butterfat percentage of Jersey cows*

Item	h^2	e^2	m	m^1	d^2
Hypothesis (1):					
Milk yield	0.56	0.10	0.04	−0.21	
Butterfat percentage	.83	−.01	.01	.09	
Hypothesis (2):					
Milk yield	.96		.04	.49	0.41
Butterfat percentage	.79		.01	.04	−.05
Hypothesis (3):					
Milk yield	.53	.09	.13	−.28	
Butterfat percentage	.83	−.01	.01	.08	

The analyses of the data on the interrelation of the daughters', parents', and sisters' milk secretion allow us to form some estimate of the effects of inheritance, environment, and differences in dominance on the observed variation in milk yield and butterfat percentage. The analytical material tabulated presents the extreme hypotheses. Possibly these hypotheses could be modified and combined in all gradations between these extremes. The results from such combinations would be intermediate, however, so that conclusions reached on the basis of this material would be essentially correct for such intermediate hypotheses.

The conclusions reached by hypothesis (1), assortive mating based on somatic resemblance and genetic factors without dominance, and (3), assortive mating genetic and no dominance of factors, are essentially the same, both qualitatively and quantitatively. Variations in heredity accounted for about half of the observed variations in milk yield and four-fifths of the observed variations in butterfat percentage. Environmental variations which have a common effect on the sisters' milk yield account for but 10 percent of the variation. On general

grounds this slight effect of environment seems too little, but such may not be the case since the cows which are to make the Registry of Merit are apt to have a more uniformly good environment than possibly the majority of cows in the breed. The minus value of the correlation between the genotypes of the sire's mates appears to be contrary to what would be expected for the Jerseys since these mates would tend to be positively correlated on two grounds, namely, that they are apt to be related genetically, and that what selection is practiced is in the direction of making the cows similar in production. This contradiction is further emphasized by the correlation actually observed for the milk production ($r = +0.17$) and butterfat percentage ($r = 0.15$) of the sire's mates. A fairly large probably error is to be attached to the -0.28 observed, however, so that it may not be significant. Other than this discrepancy, the results of both analyses look quite reasonable.

Wide crosses of low and high milk-producing breeds have furnished definite evidence of the partial dominance of high milk production (*6*, *8*, *9*, *10*, *19*). If partial dominance does exist we should expect that hypothesis (2) would lead to a fair portrayal of the conditions in the breeding of these Jerseys. This analysis indicates that heredity determines 96 percent of the variation in milk yield and 79 percent of that in butterfat percentage. The assortive mating coefficients between the sire and cows to which he is bred are low for both the milk yield and butterfat percentage. The correlation for the sire's mates is positive and fairly large for milk yield, a fact that might be expected from a general knowledge of the Jersey breed. The same correlation for the butterfat percentage is low. These correlations, on the whole, agree better with those observed, 0.17 and 0.15, than in the two previous hypotheses. The effect of the dominance ratio is rather high. It does agree with the data on the crosses of breeds widely different in milk yield in showing more effect of dominance on milk yield than on butterfat percentage.

The probability seems to be that the hypothesis which would most nearly meet the conditions in these Jerseys is intermediate between those discussed. If this is so, it may be concluded that the inheritance of the cow controls between 50 and 70 percent of the variation in milk yield and between 75 and 85 percent of the variation in butterfat percentage. The effect of environment on milk yield is 5 to 10 percent. There is little relation between the sire's genotype and the genotypes of the cows to which he is bred, but there is a fair relation between the genotypes of these cows. Finally, the effect of dominance of genes for milk yield is fairly well marked, whereas that for genes for butterfat is small.

Heredity is even more prominent in the results from a similar analysis of the relations between the granddaughters, grandparents, and various cousin combinations. These analyses show that heredity accounts for 78 percent of the variation in milk yield and 95 percent of the variation in butterfat percentage. The effect of environment on the milk yield of the cousins was 13 percent, whereas that for the butterfat percentages was but 2 percent.

The genetic correlations between the genotypes according to hypothesis (1), assortive mating somatic without dominance, and hypothesis (3), assortive mating genetic without dominance, lead to much the same values. Determined on the basis of hypothesis (1),

the parent and offspring correlations for milk yield and butterfat percentage are 0.54 and 0.51, respectively, the sister correlations are 0.52 and 0.50, respectively, and the half-sister correlations are 0.22 and 0.28. Determined on the basis of hypothesis (3), the same correlations are 0.56 and 0.51 for the parent and offspring and sisters and 0.25 and 0.28 for the half sisters. Again there is remarkable agreement between the results, either hypothesis apparently being adequate to explain them. The view consequently appears justified that the variation in the milk yields of these Jersey cows is largely due to heredity, environment playing but a small part. The theory that some of the factors for high milk yield show partial dominance also receives some slight support from this analysis. In the control of the variation of the butterfat percentage, heredity is the major factor, environment or dominance having little effect.

DISCUSSION

In the preceding section an attempt has been made to give definiteness to the terms "inheritance of milk yield" and "butterfat percentage" insofar as they relate to Jersey Registry of Merit cattle. The multiplicity of factors which have been shown to affect milk secretion have sometimes been allowed to cloud this issue. While the milk record of a cow is presumably the product of such independent characters as body constitution, good mammary-gland capacity, adequate secretory tissue, disease resistance, and breeding ability, and such environmental variables as adequate food for the full expression of the animals' inherent constitution for growth and production (*15, pp. 512–513*), so are most other inherited characters.

From the data in this paper it has been possible to separate roughly the variation of environment and the variation of the inheritance as they have influenced the milk secretion of these Jersey Registry of Merit cows. The analyses of these effects lead to the conclusion that the variations in feeding, care, and management of the cows, insofar as they are common to such close relatives as sisters, are much less important than those of heredity. The effect of the environment which is common only to the cow herself is more important than the environment common to sister cows. For milk yield the inheritance of the cow was found to control 50 to 70 percent of the variation in production; the environment common to sisters, 5 to 10 percent, and factors (some hereditary, such as dominance, and some environmental) common only to the cow herself, 20 to 45 percent. For the butterfat percentage the inheritance controls 75 to 85 percent of the variation, the environment common to sisters has no apparent effect, and the influences common only to the cow herself control 15 to 25 percent. Similar data obtained by the writer (*11, 13, 14*) on the Holstein-Friesian and Guernsey breeds led to similar conclusions.

The contribution which inheritance makes to the variation in the milk yields of these Jersey cows may itself be separated and the parts contributed by the various ancestors may be allotted to them. Individually considered, the immediate parents are found to contribute most, the effect of the sire's and dam's contribution to the daughter's milk secretion being essentially the same. No evidence that one parent consistently dominated the production of the offspring was found. The level of production which any cow has is the

resultant of the interaction of the genes transmitted to it by its parents. The variation in the productions of the daughters of any pair of parents seems to be accounted for by the chance at segregation and fertilization of the factors contained in the daughter's sire and dam. The parents' genetic constitution thus has an average value for milk production as well as a prescribed range which it may transmit to its progency, the factors transmitted by the male having an equal effect or "potency" with those transmitted by the female. This conclusion does not mean, as some have interpreted it, that the offspring of any given mating will have daughters whose milk yield will take a single value, a mean between the two parental productions; chance at maturation and segregation of the genes precludes that. The milk yield of the daughters, provided they are sufficient in number, will rather approach an average value determined by the genes possessed by the two parents, and will have a variation fixed by the segregation of their parents' heterozygous genes.

The correlation coefficients between the milk production of daughters and dams are 0.30 ± 0.01 for milk yield and 0.42 ± 0.01 for butterfat percentage. These coefficients are comparable to those for the Holstein-Friesian, 0.50 ± 0.02 for milk yield and 0.41 ± 0.02 for butterfat percentage, and for the Guernsey, 0.36 ± 0.02 and 0.42 ± 0.02. Similar daughter-dam correlations on butterfat yield have been noted by Turner (*23*) for Guernseys, 0.30, and for Jerseys (*24*), 0.35 ± 0.01; by Gifford as reported by Turner (*24*), for Ayrshires, 0.25 ± 0.02; Agar (*3*), for the Red Poll, 0.24 ± 0.06, and by Copeland (*4*) for Jerseys, 0.40 ± 0.03. Heizer (*17*), studying the records of a pure-bred Ayrshire herd, obtained daughter-dam correlations of 0.51 ± 0.03 for milk yield and 0.59 ± 0.02 for butterfat percentage. While certain of these correlation coefficients are statistically different from one another, their general trend points to a correlation between the daughter-dam milk yields or butterfat percentages of the different groups of dairy cattle of 0.4.

In a study of the daughter-dam correlations for eight different measurements of conformation in Jersey cattle (*15*), correlations of 0.37 were obtained between their estimated weights; 0.40 between their measured height at withers; 0.32 depth at withers; 0.42 heart girths; 0.35 paunch girths; 0.59 width at hips; 0.49 body length; and 0.64 rump length. These correlations, in contrast to those for milk yield, are influenced materially by a large assortive mating between the sizes of sire and dam. The data considering this assortive mating are comparable with those for milk yield and butterfat percentage as presented above.

Data quite similar to those contained in table 9 have been presented for Scottish Ayrshires (*20, 21, 22*). These data show the variance of the differences in production of the given pairs of ancestors rather than the correlation coefficients, a measure which leads to identical conclusions with the intraclass correlations. These variances for the butterfat percentage of granddaughters having A as the common grandparent are practically the same as those for granddaughters having C as the common grandparent. This fact is interpreted as showing that butterfat is autosomal in its inheritance. The earlier data on the butterfat percentage of the Holstein-Friesian and Guernsey breeds (*11, 13, 14*) have likewise been interpreted as leading to the same conclusion. The Jersey data in table 9 support this view.

The variances in milk yields of these Ayrshires with A as the common grandparent are twice as large as those with C as the common grandparent. From this fact it is suggested that sex-linked factors for milk production may be present. There are, however, distinct contradictions to this interpretation within the data when taken as a whole. Data on the Holstein-Friesian cousins do lend some support to the sex-linked theory, although here, too, contradictions exist. The data in table 9 do not support the sex-linked hypothesis for the Jersey Registry of Merit cows. Similar data on the Guernsey breed likewise lend no support to it.

The data on the grandparents of these Jersey Registry of Merit cows show that, considered individually, the correlation between their milk yields and butterfat percentages and those of their granddaughters is only about half that of a parent production correlated with the production of the daughter. These grandparental correlations average about 0.2 while those of the parents average 0.4. In forecasting the record of a daughter, the record of a parent is four times as accurate as that of a grandparent, if we think of the degree of control as the square of their correlation coefficients. When the record used for forecasting a future cow's milk yield is that of a grandparent, the hazard evidently becomes much greater. This fact is emphasized in a recent paper by Edwards (*5*) in which the transmitting ability of a group of sires is compared. The production records of the dams of three sires in the same herd were given. The sire whose daughters had the largest production had the dam of lowest milk yield. His daughers' milk yield was 1,000 pounds higher than that of their dams, while that of sire 2's daughters was 4,350 pounds less than their dams, and that of sire 3's daughters was 3,510 pounds less. While this is a selected, and therefore striking case, not comparable with the average result, it is by no means unexpected in view of the grandparental correlations derived in this paper and in former papers as cited above. It seems to emphasize the fact that the difficulties surrounding the selection of a sire almost require that the constructive breeder raise and test his breeding bulls in his own herd.

The data in this paper demonstrate the inheritance of both the quantity of secretion which a gland manufactures and the proportion of a specific constituent of this secretion. An active gland may thus secrete a large volume of fluid, but this fluid may have a fairly wide variation in the percentage composition of its constituents. Similarly a gland of less active appearance may secrete a fluid which is highly concentrated, making its total secretion of the given constituent even greater than that of the apparently more active gland.

SUMMARY

This paper presents a study of the inheritance of milk yield and butterfat percentage in about 14,000 Jersey Registry of Merit cattle. The records of production are all of 364 to 366 days in length and are corrected to the production expected at maturity as judged by the cows' record obtained at another age. Parent and offspring, grandparent and offspring, sistership and cousinship correlations are presented. These correlations show that inheritance accounts for about half the observed variation in milk yield and four-fifths of the observed variation in butterfat percentage. The environmental

variations which exist in these Jersey records account for little of the variation in the butterfat percentage and for only 10 percent of the variation in the milk yield. Dominance, assortive mating, and environmental variation common only to the cow herself account for the rest of the fluctuations in the yields and butterfat percentages.

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INOCULATIONS SHOWING THE WIDE HOST RANGE OF BOTRYOSPHAERIA RIBIS[1]

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INTRODUCTION

The organism, *Botryosphaeria ribis*,[2] which causes the cane blight of currant, *Ribes rubrum*, *R. americanum*, *R. nigrum*, and *R. aureum*, has a wide distribution. It is found in parts of the United States, Canada, Hawaii, Palestine, Algeria, Tunisia, and Australia. It is omnivorous in its host relations and has been found on plants belonging to a number of different species.

HISTORY AND HOST RANGE

The importance of *Botryosphaeria ribis* as a pathogene on currant and gooseberry, *Ribes grossularia*, was demonstrated by Grossenbacher and Duggar (*7*).[3] They described the perfect stage as *B. ribis*. The imperfect stage, which is common on most hosts, is a *Dothiorella*. Later, Shear, Stevens, and Wilcox (*17*) verified their work and studied critically (using over 3,000 cultures) the closely related fungi collected from 26 different hosts.

Shear, Stevens, and Wilcox (*18*) report 17 species of plants in the United States as hosts of *Botryosphaeria ribis*. Stevens (*20*) gives almost the same list, with additional species. Stevens and Shear (*22*) found *B. ribis* to be relatively scarce in the Hawaiian Islands. They made less than 30 collections, and list its occurrence on 14 species of plants. They grew cultures of the fungus from 15 of their collections, and proved the pathogenicity on currants of 5 of these cultures selected at random.

Saccardo (*16*) lists and describes 72 different species of the genus *Dothiorella* that were found on 54 different genera, and on a greater number of species.

Fawcett (*1*, *2*) found a species of *Dothiorella* attacking limbs of the English walnut, *Juglans regial*, named the disease melaxuma, and discovered that the arroyo willow, *Salix lasiolepis* Benth., had typical cankers caused by the same fungus. This fungus, from its imperfect form, was provisionally determined as *Dothiorella gregaria* Sacc. which is now believed to be identical with the *Dothiorella* stage of *Botryosphaeria ribis* on currant and gooseberry, as described by Grossenbacher and Duggar (*7*). A decay of citrus fruits, especially of lemons, is listed by Fawcett (*5*, *pp. 412–414*) as caused by *B. ribis*. He reports *Dothiorella* sp. as girdling and killing large citrus branches, in Italy (*3*, *p. 132*), in Tunisia (*4*, *p. 208*), and in Algeria (*4*, *p. 244*). Reichert and Hellinger (*15*) record *B. ribis* as the cause of decay of

[1] Received for publication Mar. 16, 1934; issued September, 1934. Paper no. 293, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] The name *Botryosphaeria ribis* is used in this paper for strains agreeing with *B. ribis chromogena* Gros. and Dug.

[3] Reference is made by number (italic) to Literature Cited, p. 475.

oranges, and as the cause of bark lesions on citrus in Palestine. Shear, Stevens, and Wilcox (*17*), in 1917, inoculated grapefruit with the currant cane-blight organism and produced a typical rot. Stevens and Jenkins (*21*) studied a blight on roses and a decay of the fruit of the horsechestnut and proved that it was caused by *B. ribis*. Putterill (*14*) describes a canker of the apple tree in South Africa that was caused by a fungus for which he proposed the name *Botryosphaeria mali*. Shear, Stevens, and Wilcox (*17*) believe that this fungus is not different from *B. ribis*. Fenner (*6*) describes a rot of apples caused by *B. ribis*. Horne (*8, 9, 10, 11, 12*) finds a *Dothiorella* to be one of the causes of the spoilage of mature avocado fruits. It is also believed to be the same species as *B. ribis*. Stevens (*19*) reports a species of *Botryosphaeria* (probably *B. ribis*) on avocado from Cuba.

The following are some of the hosts attacked by *Botryosphaeria ribis*, as reported in the literature: *Acalypha* sp. (*22*), *A. marginata* (*20*); *Aesculus hyppocastanum* (*20, 21*); *Aleurites moluccana* (*22*); *Amygdalus* sp. (*18*), *A. persica* (*20*); *Baccharis* sp. (*18, 20*); *Berberis sp.* (*20*); *Cassia* sp. (*20*); *Citrus sinensis* (*5*), *C. limonia* (*5*); *Eucalyptus* sp. (*18, 22*); *Gossypium* sp. (*18*), *G. hirsutum* (*20*); *Hibiscus sabdariffa* (*22*), *H. tiliaceus* (*22*); *Hicoria pecan* (*20*); *Juglans* sp. (*18*), *J. regia* (*1, 2*); *Laguncularia* sp. (*18*), *L. racemosa* (*20*); *Leucaena glauca* (*22*); *Liriodendron* sp. (*18*), *L. tulipifera* (*20*); *Liquidambar* sp. (*18*), *L. styraciflua* (*20*); *Magnolia* sp. (*20*); *Mangifera indica* (*22*); *Melia* sp. (*18*); *Pandanus odoratissimus* (*22*); *Persea* sp. (*8, 9, 10, 11, 12, 20, p. 208*); *Pipturus albidus* (*22*); *Poinciana* sp. (*18*), *P. pulcherrima* (*20*); *Psidium guajava* (*22*); *Pyrus malus* (*6, 14, 17*); *Quercus* spp. *18, 20*); *Ribes* sp. (*7, 17, 20*); *Ricinus* sp. (*18*), *R. communis* (*20*); *Rosa setipoda* and other hybrids (*21*); *Rubus* sp. (*19*) raspberry; *Sambucus* sp. (*18, 20*); *Salix* spp. (*20*); *Schinus molle* (*22*), *S. terebinthifolius* (*22*); *Vitis* sp. (*18*); *Wikstroemia phillyreaefolia* (*22*).

The occurrence in California of a similar *Dothiorella* on Citrus; avocado, *Persea americana;* and walnut *Juglans* sp.; and its isolation from *Cocos plumosa* Hook. suggested cross-inoculation experiments on these hosts with strains isolated from these four hosts. The plan was later modified and made to include inoculations on species of 25 different genera.

MATERIAL AND METHODS

Two strains of *Dothiorella* were isolated from English walnut, *Juglans regia*. One strain was obtained from the pycniospores on a dead shoot, the other from an active melaxuma canker. Cultures of the latter usually caused larger lesions than any other strain tested. The avocado strain was furnished by W. T. Horne, and was isolated from a Fuerte avocado secured from the coastal region. The citrus culture was supplied by H. S. Fawcett, and was isolated from lemon bark. The palm strain was isolated by L. J. Klotz from *Cocos plumosa*.

Botryosphaeria ribis is probably of rare occurrence on any of the Palmaceae as this is the first time that the disease has been found in California. This diseased specimen of *Cocos plumosa* was growing in a city lawn which had been sprinkled with a spray that splashed the water on the trunk and gave exceptionally favorable conditions for fungus infection. The palm was of good size with a trunk at base about 1.5 feet in diameter. These natural infections consisted of

numerous black lesions that were 1 to 6 inches in diameter and 1 to 1.5 inches deep (pl. 3, *A*). They were for the most part arranged in a straight line that extended from near the base of the palm to a height of about 6 feet, but some other lesions were found at other points without any definite arrangement.

The method of inoculation consisted in placing a pure culture of the organism from glucose-potato agar in injuries in the bark of trees growing in the open. Uniform wounds were secured by making cylindrical holes with a cork borer, 7 mm in diameter. After the insertion of the fungus, the excised bark was replaced and the whole wrapped with nurseryman's tape. This method of inoculation is similar to that described by Klotz and Fawcett (*13*) and Wright (*23*). On small twigs a slight tangential oblique cut into the tissue was made, the inoculum placed in the incision, and the twig wrapped with tape. All the inoculations except some later ones on the Palmaceae were protected with nurseryman's tape. These other inoculations on the Palmaceae were made by placing the inoculum over the injury and moist cotton over the inoculum. Then all was wrapped with paraffin paper whose ends were fastened to the plant tissue with tape. Control experiments were run at the same time as the inoculations. They were made on the same or an adjacent twig, were wounded and protected in the same manner as the inoculations.

INOCULATION AND RESULTS

Inoculations with controls were made on different species of Rosaceae, Juglandaceae, Palmaceae, and on *Citrus*, *Persea* (avocado), and other genera. The results, although somewhat variable, were consistent in that nearly all the species showed some evidence of susceptibility. Some results were negative. These, before being considered as conclusive, should be confirmed by repeating the inoculations. The size of the diseased areas was judged by measuring the longest radius of blackened or discolored tissue, in millimeters, from the margin of the infection court. A radius less than 5 mm does not have much pathogenic significance. The results of inoculation on the hosts used are tabulated in tables 1 to 4 under the family names Rosaceae, Juglandaceae, and Palmaceae, the genera *Citrus* and *Persea*, and a miscellaneous group of species. The species that have not been reported in literature as hosts of *Botryosphaeria ribis* are marked in tables with an asterisk.

TABLE 1.—*Results of inoculations with Botryosphaeria ribis on plants belonging to Rosaceae, Juglandaceae, and Citrus*

ROSACEAE

Hosts inoculated [1]	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	*Number*	*Number*	*Millimeters*
*Almond (*Prunus communis*)	Walnut	35	4	4	25–50
Do	Avocado	61	6	3	10–25
Apple (*Malus sylvestris*)	Walnut	33	8	7	3–90
Do	Avacado	21	4	4	30–45
Do	Palm	25	3	2	15–25
Apple (fruit)	Walnut	16	2	2	20–30
*Apricot (*Prunus armeniaca*)	do	40	10	6	15–35
Do	Avacado	40	8	4	25–50

[1] Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (*).

TABLE 1.—*Results of inoculations with Botryosphaeria ribis on plants belonging to Rosaceae, Juglandaceae, and Citrus*—Continued

ROSACEAE—Continued.

Hosts inoculated	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	*Number*	*Number*	*Millimeters*
Blackberry (*Rubus* sp.)	Walnut	10	4	3	20–30
Blackberry (Mammoth)	Citrus	15	3	2	15–20
Blackberry (*Rubus* sp.)	Avocado	10	5	3	10–20
Do	Palm	25	3	3	5–10
*Cherry (*P. avium*)	Walnut	36	7	7	10–70
Do	Avocado	62	3	3	[2]
*Cherry (*P. serotina*)	Walnut	25	2	2	40–50
**Cotoneaster pannosa*	do	40	3	2	30–90
Do	Avocado	40	3	3	10–30
Heteromeles arbotifolia	Walnut	22	3	3	20–40
Do	Avocado	22	3	3	5–25
*Loquat (*Eriobotrya japonica*)	Walnut	22	3	2	20–30
Do	Avocado	24	3	2	15–25
Peach (*Amygdalus persica*)	Walnut	23	3	3	25–60
Do	Avocado	23	4	3	30–35
Do	Citrus	27	4	3	5–10
*Pear (*Pyrus communis*)	Walnut	36	3	3	70–80
*Plum (*Prunus domestica*)	Avocado	48	3	2	25–30
*Plum (*P. salicina*)	Walnut	50	4	4	30–100
**Pyracantha gibbsii*	do	20	3	3	20–40
P. yunnanensis	do	20	3	3	5–15
Rosa sp. Lady Hillingdon	do	18	3	3	15–35
Rosa, Maman Cochet	Avocado	18	3	3	10–20
Rosa sp	Palm	25	3	3	10–15
*Quince (*Cydonia oblonga*)	Walnut	25	7	6	20–100
Do	Avocado	25	4	3	10–25
Do	Palm	25	2	2	10
Do	Citrus	30	2	0	

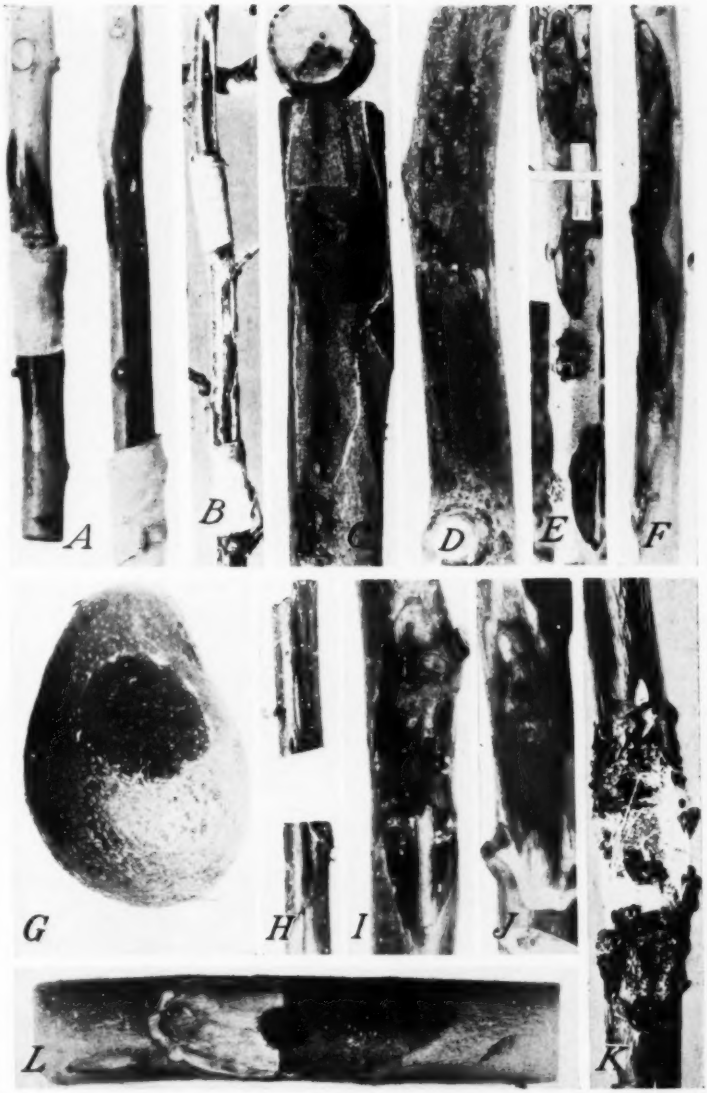
JUGLANDACEAE

**Juglans nigra*	Walnut	45	12	3	5–10
Do	Avocado	36	6	0	
Do	Citrus	33	3	2	5–5
J. regia	Walnut	45	4	4	30–40
Do	Avocado	45	5	5	10–25
Do	Palm	25	3	3	25–60
**J. californica*	Walnut	45	5	4	5–20
Do	Avocado	45	2	0	
**J. hindsii*	Walnut	45	4	4	5–10
Do	Avocado	45	3	2	4–5
**J. major*	do	30	3	3	10
Do	Walnut	30	3	3	20–30
**J. sieboldiana*	Avocado	45	3	2	5–10
Carya pecan	do	24	2	2	3–5
Do	Walnut	60	2	2	10–50
**Pterocarya fraxinifolia*	Avocado	20	2	2	3–5
Do	Walnut	20	5	2	5–7

CITRUS SHOOTS AND FRUITS

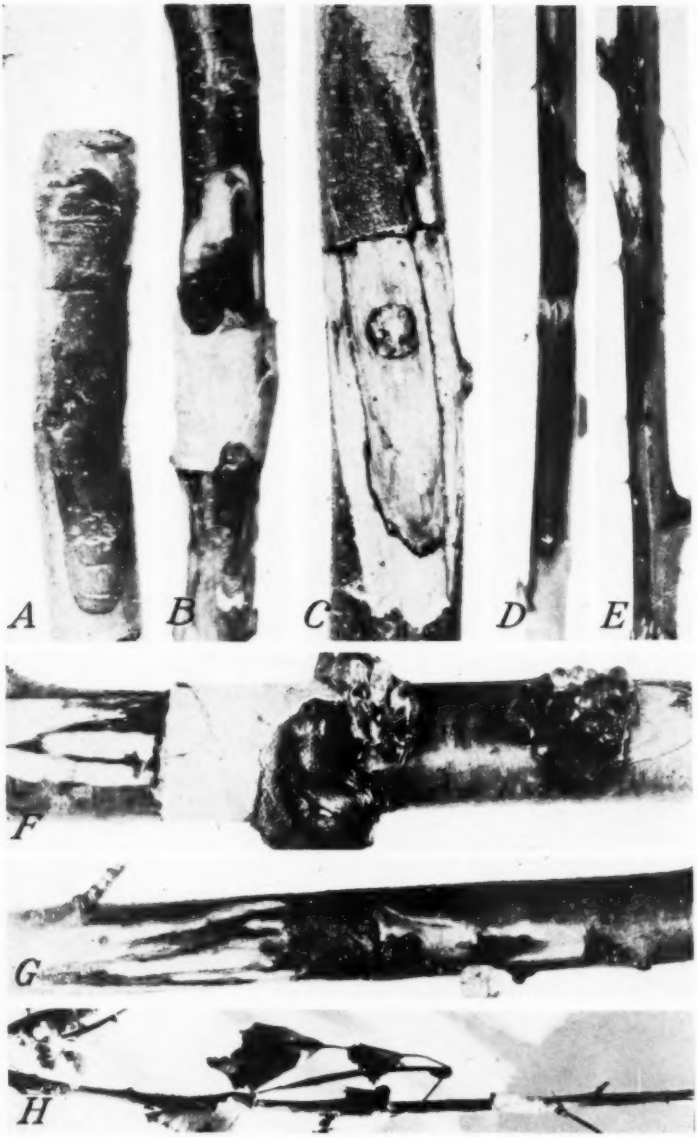
Citrus shoots:					
Citrus sinensis, Washington Navel	Walnut	45	4	4	5–7
C. sinensis, Valencia	do	13	6	6	40–70
Do	Palm	25	2	2	5
C. grandis, Marsh	Walnut	13	3	3	15–50
Do	Avocado	30	3	3	30–35
C. limonia, lemon	Walnut	26	3	3	20–60
Do	Avocado	45	4	4	10–15
C. aurantium, sour	Citrus	27	3	2	3–5
Do	Walnut	36	10	4	10–15
Do	Avocado	36	4	0	
Citrus fruits:					
Citrus sinensis, Washington Navel	Walnut	10	1	1	50
Do	Avocado	12	1	1	15
C. sinensis, Valencia	Walnut	12	1	1	70
Do	Avocado	12	1	1	50
C. grandis, Marsh	Walnut	12	2	2	70–140
Do	Avocado	12	2	2	25–70
C. limonia, lemon	Walnut	12	2	2	25–50
Do	Avocado	12	2	2	25–50

[2] Girdled.



ARTIFICIAL INOCULATIONS ON DIFFERENT HOSTS WITH BOTRYOSPHAERIA RIBIS FROM CITRUS, AVOCADO, AND WALNUT.

A, Avocado strain on stems of *Persea americana* (avocado). *B*, Walnut strain on *Corylus avellana* L. (filbert). *C*, Walnut strain on *Eucalyptus* sp. after 50 days. *D*, Carya pecan inoculated with walnut strain. *E*, Walnut strain on *Juglans major* after 60 days. *F*, Walnut strain on *Citrus grandis*, Marsh grapefruit. *G*, Fruit of Guatemalan avocado, *Persea americana*, inoculated with walnut strain of *Dothiorella* sp. in contact without wounds. *H*, Walnut strain on *Castanea* sp. after 27 days. *I*, Inoculation on *J. regia* with walnut strain. *J*, Walnut strain on *Annona cherimola*. *K*, Valencia orange, *C. sinensis*, inoculated with walnut strain May 5, photographed October 5, 1933. *L*, Walnut strain on *Olea europaea* after 50 days.



ARTIFICIAL INOCULATION ON RIBES VULGARE AND PLANTS OF THE ROSACEAE WITH CULTURES OF BOTRYOSPHAERIA RIBIS ISOLATED FROM WALNUT, AVOCADO, AND CITRUS.

A, Walnut strain inoculated in twigs of *Pyrus malus*, photographed after 32 days. *B*, Walnut strain on *Prunus armeniaca* (Royal apricot) after 64 days. *C*, Walnut strain on *Cydonia oblonga* Mill. (quince), photographed after 51 days. *D*, *Rosa* sp. (Lady Hillingdon) inoculated with walnut strain, photographed after 25 days. *E*, Inoculation with walnut strain of *Dothiorella* sp. on Mammoth blackberry *Rubus* sp. *F*, Walnut strain inoculated on sweet cherry, *Prunus avium*, 60 days. *G*, Walnut strain on *P. serotina* Ehrh., photographed after 22 days. *H*, currant, *R. vulgare*, 64 days after inoculation with walnut strain of *Dothiorella* sp.

The genera of the Rosaceae (table 1) inoculated were *Cotoneaster*, *Cydonia*, *Eriobotrya*, *Heteromeles*, *Rosa*, *Rubus*, *Prunus*, *Pyrus*, and *Pyracantha*. In varieties of *Prunus domestica*, *P. avium*, *P. communis*, and *P. persica*, definite infected areas developed. A large amount of gum was often present, whether the results were positive or negative (*Citrus*, pl. 1, and *Prunus*, pl. 2). Two varieties of rose, Lady Hillingdon and Maman Cochet, were especially susceptible and developed diseased areas having a radius of 10 to 35 mm in 18 days (pl. 2, *D*). On Mammoth blackberry, positive lesions 10 to 30 mm in radius were formed (pl. 2, *E*). The apple and pear readily developed cankers 30 to 80 mm in radius in about 30 days (pl. 2, *A*). The loquat, *Eriobotrya japonica*, the quince, *Cydonia oblonga*, the California Christmasberry, *Heteromeles arbutifolia*, and firethorn, *Pyracantha gibbsii* and *P. yunnanensis*, were all readily infected. The quince (pl. 2, *C*) and loquat were especially susceptible and had infected areas 20 to 50 mm in radius in 30 days.

Of the Juglandaceae tested, the various walnuts *Juglans regia*, *J. californica*, *J. hindsii*, *J. major* (pl. 1, *E*), and *J. sieboldiana* were all susceptible and had diseased areas with a radius of 5 to 40 mm. *J. regia* had the largest canker, ranging from 30 to 40 mm in radius in 45 days (pl. 1, *I*). The diseased areas on the other species were 5 to 20 mm in radius. *J. nigra* showed negative results in 15 inoculations and small lesions (5 mm radius) in 5 inoculations. The inoculations on *Pterocarya fraxinifolia* were of small size. On *Carya pecan* the infected areas were 10 to 15 mm in radius in 24 days, increasing to 50 mm after 60 days (pl. 1, *D*).

Inoculations were made on shoots of the following species of *Citrus: C. sinensis*, Washington Navel and Valencia orange (pl. 1, *K*); *C. grandis*, Marsh grapefruit (pl. 1, *F*); *C. limonia*, the lemon; and *C aurantium*, the sour orange. Of these, the lemon was the most susceptible, having cankers 20 to 60 mm in radius in 10 days. The sour orange was the most resistant, with small positive lesions 10 to 15 mm in radius in the bark and wood one-half to three-quarter inch in diameter; on older, large wood the results were negative. The sweet orange (Washington Navel and Valencia varieties) was next to the lemon in susceptibility, and had developed much gum and diseased areas of 40 to 70 mm in radius in 15 days. Marsh grapefruit shoots were readily infected, developing cankers 70 mm in radius in 15 days. The fruits of all the species, including lemon, Marsh grapefruit, and Valencia, and Washington Navel were readily infected when mycelium of the fungus was placed in a wound or on the stem end of fruits when they were kept in a moist chamber.

With the walnut strain, 25 inoculations on *Persea americana*, the avocado (table 2), developed small-sized lesions having a 2 to 5 mm radius. With the avocado strain, 11 inoculations agreed in size with those given above, while 10 other inoculations with the avocado strain were larger and produced diseased areas 10 to 50 mm in radius (pl. 1, *A* and *G*).

Fruits of avocado of various degrees of maturity were inoculated with *Botryosphaeria ribis* without being removed from the tree. Young fruits 1 to 1½ inches in diameter, when inoculated through a wound made by a 4-mm cork borer, developed spots having a radius of 4 to 5 mm (pl. 1, *G*) in 12 days. Other inoculations made in the

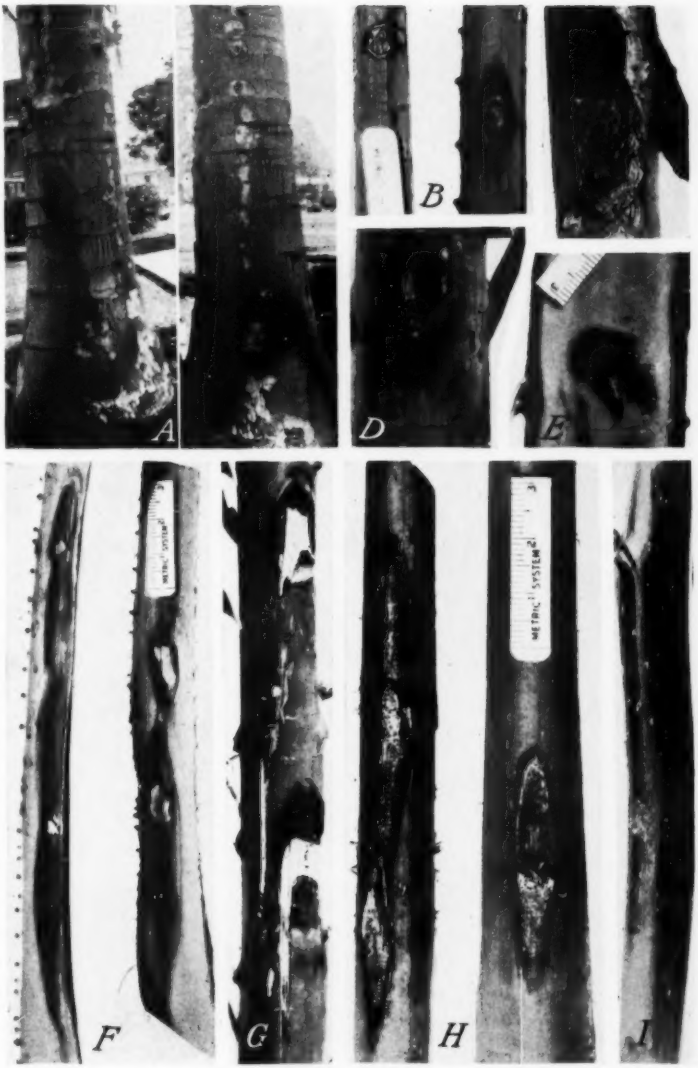
same way and on the same kind of avocado fruit showed no invasion of the tissue. Fruit of a nearly mature, green-fleshed Guatemalan avocado, inoculated through a wound made by a cork borer, developed an infected spot with a radius of 15 to 20 mm in 7 days. Young fruits of Puebla and Blake avocado inoculated without wounds by placing the mycelium growing in agar on the surface and covering it with nurseryman's tape, gave definite spots 8 to 15 mm in radius in 6 to 10 days. Diseased areas with a radius of 30 to 35 mm were also produced in a Guatemalan fruit when inoculated by placing mycelium on the surface. These results are not surprising since the avocado fruit has numerous natural openings or lenticels through which the mycelium might readily pass.

TABLE 2.—*Inoculations through wound (unless otherwise indicated) in stems and fruit of Persea americana (avocado) with cultures of Botryosphaeria ribis*

Tissue or kind of fruit inoculated	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	*Number*	*Number*	*Millimeters*
Shoots	Walnut	18–45	19	15	2–5
Do	do	18–45	3	3	5–10
Do	Avocado	18–22	11	11	2–5
Do	do	25	5	5	5–35
Do	Citrus	25–33	8	7	20–25
Do	Palm	25	1	1	5
Guatemalan fruit	Walnut	7	3	3	20–25
Do	Avocado	7	3	3	15–20
Do [1]	Walnut	7	3	3	18–15
Puebla fruit	do	7	2	2	15–12
Do	Avocado	6	2	2	4–5
Blake fruit [1]	Walnut	6	4	4	8–10
Do	Avocado	6	1	1	10–15
Do	Citrus	12	1	1	5

[1] Fruit inoculated while attached to tree by placing mycelium in contact with unwounded surface and covering with nurseryman's tape.

Successful inoculations through wounds with a culture of *Botryosphaeria ribis* isolated from *Cocos plumosa* were made in the following fruits: Apple, lemon, olive, and tomato. Inoculations on woody stems of hosts that had proved to be susceptible to the avocado, citrus, and walnut strains of *B. ribis* were also infected when inoculated with the palm strain of *B. ribis*, and developed into positive lesions that were similar in size, shape, and other characteristics to those made by the other three strains of *B. ribis*. The results from these inoculations (the number of positive results and the radius in millimeters) are given in tables 1 to 4. The inoculations of Palmaceae (pl. 3, *B–F*) with avocado, citrus, palm, and walnut strains are listed in table 3, and were made during December and January, which during this season were not excessively rainy. The amount of rain falling at Riverside, Calif., during December 1933, was 1.66 inches, and during January 1.01 inches.



NATURAL LESIONS ON COCOS PLUMOSA AND INOCULATIONS WITH THE PALM AND CITRUS STRAINS OF BOTRYOSPHAERIA RIBIS.

A, Two views of *Cocos plumosa* having natural lesions caused by *Botryosphaeria ribis*, with surface of palm cut away in some lesions to show the blackened tissue. *B*, *Cocos bonnetti* showing control and lesion formed from inoculation with palm organism, photographed after 5 months. *C*, Lesion on apple, inoculated with palm organism, showing pycnia that have formed, photographed after about 6 months. *D*, Lesion on petiole of *Phoenix canariensis* after 60 days. *E*, Petiole of *Washingtonia filifera* lesion after 60 days. *F*, Inoculations on *Erythea edulis*, two lesions coalesced at left having been caused by the palm strain of *Botryosphaeria ribis*, the lesion at the right having been caused by the citrus strain. *G*, Lesion on petioles of date palm, *Phoenix dactylifera*, after 60 days. *H*, Inoculations on *Cocos plumosa*, showing pycnia that have been formed, photographed after 60 days. *I*, Artificial lesion on *Juglans regia* caused by palm organism after 35 days.

TABLE 3.—*Inoculations through wounds in the petioles of the Palmaceae with cultures of Botryosphaeria ribis*

[Results after 60 days]

Hosts inoculated [1]	Source of culture	Inoculations	Positive results	Radius of infected area
		Number	*Number*	*Millimeters*
**Cocos bonnetti*	Palm	3	1	10
Do	Walnut	4	3	5–10
Do	Citrus	2	1	10
**C. plumosa*	Palm	8	8	5–30
Do	Walnut	5	3	5–120 by 5
Do	Avocado	2	0	[2] 2
Do	Citrus	4	0	[2] 2
**Erythea edulis*	Palm	4	4	20–280 by 5
Do	Walnut	2	2	10
Do	Citrus	3	2	5–10
Do	Avocado	1	1	5
**Phoenix canariensis*	Palm	3	3	5–20
Do	Walnut	2	2	10–300
Do	Citrus	2	2	5
**P. dactylifera*	Palm	3	3	3–5
Do	Walnut	3	3	8–10
Do	Citrus	2	2	5–10
**Washingtonia filifera*	Palm	2	1	10
Do	Walnut	4	3	5–130 by 4
Do	Citrus	3	2	5–10

[1] Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (*).
[2] Negative.

TABLE 4.—*Miscellaneous species inoculated with Botryosphaeria ribis*

Species inoculated [1]	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	*Number*	*Number*	*Millimeters*
**Annona cherimola* (cherimoya)	Walnut	15	7	7	25–55
Do	Avocado	15	6	6	5–25
**Castanea* sp.	Citrus	33	3	2	5–10
Do	Walnut	23	3	3	20–45
**Carissa grandiflora*	Avocado	17	3	2	10–20
**Ceratonia siliqua* (carob)	Walnut	22	3	3	25–35
Do	Avocado	22	3	3	5–25
**Chionanthus virginica*	Walnut	16	3	3	10–20
Do	Citrus	30	2	0	
**Corylus avellana*	Walnut	20	5	2	40–50
**Cupressus macrocarpa*	do	16	3	3	30–45
**Diospyros kaki*	do	25	4	3	5–10
D. lotus	Avocado	30	5	3	5–15
Do	Palm	25	3	3	5–10
Eucalyptus sp.	Walnut	10	3	3	50–65
Do	Palm	25	3	2	60–70
**Feijoa sellowiana*	Walnut	45	5	4	20–40
**Ficus* (Kadota fig)	do	18	3	3	3–10
**Fraxinus ornus*	do	25	3	3	15–20
Do	Avocado	25	3	2	2–10
**Juniperus procera*	Walnut	25	3	3	5–35
**Morus nigra*	do	22	2	2	5–10
**Olea europaea*	do	10	2	3	10–25
Do	Avocado	30	6	6	10–20
Do	Palm	25	2	2	5–7
**Platanus racemosa*	Walnut	16	3	3	5–35
Do	Citrus	27	3	2	5–10
**Populus fremonti*	Walnut	47	6	2	30–35
Psidium guajava	do	33	6	6	15–70
Do	Avocado	18	6	3	15–40
Do	Palm	25	3	3	35–100
**Quercus lobata*	Walnut	16	3	3	5–30
Ribes vulgare	do	90	5	2	10–20
Do	Citrus	90	2	0	
Do	Avocado	90	3	0	
Salix nigra	Walnut	25	10	10	20–40
Do	Citrus	33		2	10–25
Do	Avocado	90	2	0	
**Sapium sebiferum*	Walnut	45	3	3	15–30
**Ulmus parvifolia*	do	16	3	3	10–20
Vitis vinifera	do	33	3	3	5–6

[1] Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (*).

The results of inoculation on 20 species of miscellaneous plants are summarized in table 4. They include *Castanea* sp., chestnut (pl. 1, *H*); *Corylus arellana*, filbert; *Diospyros kaki* and *D. lotus*, persimmon; *Olea europaea*, olive; *Psidium guajava*, lemon guava; *Populus fremonti*, cottonwood; *Platanus racemosa*, sycamore; *Salix nigra* var. *vallicola*, willow; *Ulmus parvifolia*, elm; *Quercus lobata*, oak; and *Annona cherimola* (pl. 1, *J*).

The diseased areas on the olive (pl. 1, *L*), with the walnut strain had a radius of 10 to 25 mm in 10 days; those on the lemon guava a 50 to 70 mm radius in 15 days; those on *Platanus racemosa* a 10 to 40 mm radius after 16 days; those on *Quercus lobata* a 15 to 30 mm radius in 15 days; those on *Corylus arellana* a 5 to 35 mm radius in 16 days, increasing in size until they were 40 to 50 mm in radius after 60 days (pl. 1, *B*).

Consistently negative results were secured from six inoculations on each of the following species: *Casimiroa edulis* (sapota), *Zizyphus jujuba*, and *Machadmia ternifolia* (Queenland nut).

In the inoculations on woody shoots the bark and wood became invaded. The effects of the fungus were most marked on the wood, which was stained to a greater extent than the bark lesion. The extent of this wood invasion is shown in pl. 1, *C*, in the blackened V-shaped area of a section of a eucalyptus stem. Often the invasion of the organism is shown as a narrow, dark-colored strip in woody tissue that is not visible in the bark.

The controls were usually healed over at the end of the experiment in all the hosts except the Palmaceae, in which there was merely a drying of the margin of the wound. (See pl. 3, *B*, a control for an inoculation on *Cocos bonnetti*.)

The inoculations listed in tables 1, 2, and 4 were made with the palm strain in December 1933 and January 1934, and with all other strains from March to August 1933. This latter period was near the end of the rainy season and during the dry season. As shown by the records of the Citrus Experiment Station, Riverside, Calif., the amount of rain in inches was as follows: March 1933, 0.04; April, 1.28 (three showers of 0.31, 0.40, 0.42, and four light sprinkles); May, 0.30; June, 0.02; July, 0. Diseased areas developed during each of 5 months. The difference in their size and number was not significant. During July the inoculations were chiefly confined to avocado fruit, which was infected while on the tree. The temperatures, while variable, did not appear to be a controlling factor when the mycelium was placed in a wound in the tissue and sealed with nurseryman's tape.

REISOLATION OF THE FUNGUS

Botryosphaeria ribis was readily reisolated in pure cultures from small pieces of tissue cut from the outer margin of the affected areas of selected inoculations taken from most of the species listed in tables 1 to 4.

SUMMARY AND DISCUSSION

Botryosphaeria ribis is found in many of the temperate and subtropical countries. Its host range in nature includes at least 34 genera and 20 families of plants. Cultures of the *Dothiorella* stage isolated from *Juglans regia*, *Persea americana*, *Citrus limonia*, and

Cocos plumosa, when used in cross inoculations, indicate that these strains of *Dothiorella* are identical. The inoculations described in this paper were made on over 50 species of plants distributed among 39 genera and 20 families. The positive results show lesions 5 to 90 mm in radius.

The experiments show that the mycelium of the *Dothiorella* stage of *Botryosphaeria ribis* can invade the bark and woody tissue of a number of different species of Rosaceae, Juglandaceae, Palmaceae, *Citrus*, and other plants of economic importance. This suggests the possibility that some of the large number of described species of *Botryosphaeria* and of *Dothiorella*, based mainly on host relations may be the same. The evidence shows that with wounds and sufficient inoculum certain hosts are extremely susceptible, while others are apparently more resistant. In nearly all the inoculations, healthy tissue was actually invaded. With more humid conditions, larger lesions might be expected. Under natural conditions, it is probable that many of these slightly susceptible hosts would only rarely, if ever, become infected because of the lack of sufficient inoculum to break down the initial resistance.

Relatively few inoculations were made on the different hosts, and a fairly high percentage of positive results were secured. The number of inoculations, however, was too small to give any reliable evidence of the species resistant to the different California strains of *Botryosphaeria ribis*. The experiments were planned to determine the pathogenicity of the organism on different hosts rather than to show plant resistance or even to test whether it is active on some plants and not on others. Often some inoculations were very active, and others on the same branch made under the same conditions were negative. The negative results in the tables probably have no significance.

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